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Award Number: DAMD17-01-1-0110

TITLE: Molecular Determinants of Radioresponse in Prostate Cancer

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REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
Molecular Determinants of Radioresponse in Prostate Cancer

5. FUNDING NUMBERS
DAMD17-01-1-0110

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

The overall hypothesis of this project is that the radiation response of normal and cancerous prostate tissues can be correlated to the appropriate sensing and repair of DNA breaks by repair complexes following exposure to ionizing radiation. Specific aims relate to determining the interaction of DNA repair proteins in vitro using immunoflorescent confocal microscopy and biochemical DNA rejoining assays under both hypoxic and oxic conditions (given in vivo tumour cell populations). An in vivo program of prostate xenograft radioresponse is also being initiated to determine the level of DNA repair *in situ* using immunohistochemistry and immunoflorescent markers. Our studies show that terminal growth arrest rather than apoptosis is the major death pathway for irradiated prostate cancer cells and that prostate cancer cells have a DNA repair defect when compared to normal prostate epithelial cells. This appears to be related to the expression and intracellular function of the rad51 protein. Current experiments are designed to determine whether DNA protein focal interactions can predict the radioresponse of prostate xenografts and human tumors, in vivo. Our studies support the use of novel radiosensitizing compounds and predictive diagnostic assays for cancer therapy using DNA repair biomarkers.

14. SUBJECT TERMS	15. NUMBER OF PAGES
radiosensitivity, DNA repair, confocal microscopy, protein complexes, prostate cancer	72
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction:

The current funded study entitled "Molecular mechanisms of radioresistance in prostate cancer", is investigating the role of DNA break repair in the radiation response of normal and malignant prostate epithelium. The overall hypothesis of this project is that the radiation response of normal and cancerous prostate tissues can be correlated to the appropriate sensing and repair of DNA breaks by repair complexes following exposure to ionizing radiation. Specific aims relate to determining the interaction of DNA repair proteins in vitro using immunoflorescent confocal microscopy and biochemical DNA rejoining assays under both hypoxic and oxic conditions (given in vivo tumour cell populations). An in vivo program of prostate xenograft radioresponse is also being initiated to determine the level of DNA repair *in situ* using immunohistochemistry and immunoflorescent markers. These initial studies will determine the heterogeneity in fractionated response in a series of prostate xenografts as relates to DNA repair capacity, which may be translated to novel markers for radiation response in patients who receive prostate radiation therapy. The relevance of this project is that this *in vitro* to *in vivo* pre-clinical approach may derive clinical biomarkers of radiation response which can predict which patients will most benefit from radiation therapy for prostate cancer. The project will also determine the molecular mechanisms behind radiation response, in general, in prostate epithelial tissues (1,2,5)

Body:

Task 1: Initially, the first task was to primarily complete in vitro studies on PC-3; LNCaP, DU145 and normal prostate (PRSE and PREC) cells relating to DNA double strand break repair. Clonogenic radiation curves were derived for all 5 cell cultures, and complete apoptosis assays were determined in both a dose and time responsive manner. We observed that although there were differences in SF2 value for all 5 cell lines, there was little variability in radiation induced apoptosis and no evidence for dose-dependancy. This suggests that there were alternate death mechanisms for prostate cells following exposure to ionizing radiation which was determine to be post-mitotic cell death and senescence(2). This is important as it supports our grant's hypothesis that DNA repair is an important endpoint for study as it correlates more to cell survival using terminal growth arrest or cell senescence. For example, in all 5 cell lines, we found that there was an increase in the p16 superscript ink4a and $p21^{WAFWAF}$ genes at 5-9 days following 2-10 Gy of ionizing radiation. Furthermore, these cells were found to contain senescent populations which was quantitated and found to be dose responsive and correlated to their clonogenic survival (using a novel fluorescent flow cytometric proliferation assay). This work has been accepted for publication in ***Prostate Cancer and Prostate Diseases (Nature Publishing Group)*** and should be out in the fall issue of 2002 (Refer to text and figures contained in manuscript 1, Bromfield et al; appended as Appendix 1).

Task one projects were also completed for all 5 cell lines in determining the ability for the cells to repair double strand breaks, single strand breaks and DNA base damage (and oxidative damage) following ionizing radiation using the comet assay. Preliminary data with pulse field gel electrophoresis (PFGE) and continuous field gel electrophoresis

(CFGE) suggested that the results for DNA break assays correlated well to the Comet assay. As the Comet assay can give further information cell cycle distribution following DNA damage, we utilized this assay for all further experiments. We have observed a series of novel observations which currently suggests that malignant prostate cancer cells have a DNA repair defect in the repair of DNA-dsbs, DNA-ssbs and DNA-base damage and oxidative damage in relation to the two normal prostate epithelial and stromal cell cultures (4,6; see Figure 1). **This has not been reported before.** Current experiments are discerning whether or not repair is altered under oxic versus hypoxic conditions and ealry data suggest that rad51 is reduced in expression under hypoxic conditions. These studied will be completed in the next 6 months.

RNA protection and Western blot analyses suggest that that the non-homologous (ie Ku70/80/DNA-PK) and homologous(ie rad50/51/XRCC3) proteins are upregulated at the transcriptional level in the malignant prostate cells compared to normal prostate cells (see Figure 2). We therefore went onto to discern protein function in terms of protein-protein interations during DNA repair. Antibody labeling conditions and image analysis programs have been completed to utilized quantitative confocal microscopy for DNA complexes regarding RAD51, RAD50, MRE11, P95, BRCA1, BRCA2, P53, RPA, ATM and DNA-PK protein co-localization following DNA damage. We initially utilized RNA protection analyses (RPA) and western blots to show that malignant prostate cancers have an increased expression of homologous recombination-associated protein complexes, including XRCC3 and RAD51 proteins. This result was also recently reported in a publication early in 2002 by Haaf and co-workers (7).

However, more importantly, our quantitative confocal microscopy has shown that all though the 3 malignant prostate epithelial cell lines (PC-3, DU-145, LNCaP) have high levels of protein expression on western blot analysis, they do not have functional RAD51 in terms of forming nuclear complexes following DNA damage. We observed that there is a decreased incidence of ionizing radiation induced foci of RAD51 following 10 Gy of irradiation when compared to the normal cell cultures. In fact it appears, that the LNCaP cell line, shows perinuclear staining following ionizing radiation and cannot be transported into the nucleus following DNA damage. It may therefore be that there are mutations in the BRCA2 and BRCA1 genes which are responsible for complexing with the RAD51 protein, and all 5 cell lines are being sequenced for BRCA1 and BRCA2 and RAD51 in separate experiments (9). **This result has not been reported in the literature before, and may suggest that although there are increased levels of RAD51 and tumour cell lines, they may not have an increased fidelity of DNA repair if the protein cannot translocate from the cytoplasm to the nucleus.** A quantitative assessment of the DNA complexes following radiation by both RNA, protein and repair foci complex formation endpoints have been completed and a manuscript is in preparation to be submitted in the next 3 months regarding this (Fan et al., 2002; see Figures 3, 4).

Primary prostatic cultures were initiated as prostatectomy specimen cultures available through Coriell Laboratories. We were successful in culturing both the prostate stromal and the prostate epithelial cell cultures in vitro to be utilized in a radiobiology and DNA

repair experiments. DNA double stand break repair complexes have been determined in these cultures and are part of the data set contained in the manuscript in Appendix 1 and the manuscript in preparation, as noted above.

Xenografts from Erasmus University, Rotterdam were received after an MTA was signed and they were implanted into Balb/c mice for growth assays. An ethics approval for biopsies taken before and after 5 fraction (25 Gy in 5 fractions pre-operative radiotherapy) during clinical prostate radiotherapy was given by our research ethics board (REB) and this was submitted to the US Army as part of the documentation for this grant. Eight patients have been accrued to the phase 1 pilot study of preoperative radiotherapy, and we are using both pre and post radiotherapy biopsies on these patients to derive the markers related to in vivo irradiation or patient irradiation as outlined in Tasks 2 and 3. We have therefore completed all endpoints for Task 1, as a result, manuscripts are in preparation, or in press, in relation to Task 3.

Task 2: Complete in vivo radiobiologic studies on human prostate xenografts (months 12-24).

We have determined that there can be cell cycle phase specific changes in DNA-double strand break foci formation, in relation to DNA rejoining, by irradiating cells under both a synchronous and G0-G1 synchronized conditions. However it was difficult to synchronize the malignant cells completely in G1 and we only could synchronize them to a point that 90% of cells were in G1 at the time of irradiation, compared to >95% for the normal cell lines. Our data suggests that DNA-double strand break foci, including homologous recombination-associated proteins, do form in the absence of DNA replication in G1 and are dose responsive. Selected experiments looking at G1 versus S versus G2 phase of the cell cycle and DNA double strand break foci formation in malignant and normal cell lines will take place over the next 6-12 months.

Single dose experiments have been completed for the PC-3 and the selected Rotterdam xenografts, utilizing the dose of 20 Gy, and then the xenografts were removed and stained for immunohistochemical markers pertaining to p53, apoptosis related genes (bax, BCL2 and TUNEL assay as well as survivin) and DNA repair markers (RAD50, BRCA1, BRCA2, RAD50, $DNAPK_{cs}$, KU70, KU80, ATM, P21, RB, MYC and RPA). The xenograft histology was also stained for proliferation markers such as MIB-1, KI-67 and PCNA. Our early results suggest that RAD51 and RAD50 and BRCA2 increase in expression both in terms of the nuclear intensity of staining as well as a number of cells positively staining for protein following 20 Gy or irradiation *in vivo* at 24 hours (e.g. see Figure 4). **This has not been reported in the literature before (8,10)**. It is unclear whether or not the increase in staining is occurring in the oxic versus hypoxic compartments and as such we are pursuing these studies relating to relative expression within the two compartments using hypoxic markers (GLUT-1 and HIF1alpha) in separate experiments.

Growth delay will be determined in the next 12 months for fractionated, single dose in control groups of animals containing the PC-3 DU-145 and PR-346C and other

Rotterdam xenograft to complete Task 2. IHC endpoints will be completed as in the previous paragraph for all animal treatment cohorts.

Unlike *in vitro* experiments, the observation of discrete DNA repair foci using normal microscopic IHC within histologic specimens of xenografts irradiated *in vivo* have been difficult and does not have the resolution of the confocal microscopy. We are continuing to modify the microscope technology in order to do this, and also optimize our tissue fixation prior to antibody use to allow for confocal microscopy based on frozen sections. However, it may be that the relative subcellular localization of repair proteins (cytoplasmic versus nuclear), such as that we have observed in studies described in Task 1 for RAD51, may give us a unique marker for malignancy, as well as radiation response that can not solely be quantitated using the number of foci, per se.

Accrual of peri-radiotherapy biopsies from clinical patients after fraction 5 for analysis will be starting in the next 6 months, as we have altered our radiation protocol to improve IMRT-treated patients as well as a new dose for dose escalated conformal radiotherapy (change from 75.6 to 79.8 Gy). Nonetheless, six patients so far have been accrued for the preoperative radiotherapy study, and we are just in the midst of sequencing their p53 status for all 11 exons of the p53 gene. Baseline immunohistochemistry for p53, p21, TUNNEL, bax and BCL-2 have been completed. We are currently staining the Phase I pre-operative radiation study patients (i.e. post 25 Gy) biopsies to compare pre and post radiotherapy specimens for the unique markers outlined above and this should be completed within the next 6-9 months.

Task 3: (Months 18-36)

Task 3 is to primarily complete the clinical studies undergoing radiotherapy, and to determine DNA repair foci in human clinical biopsies pre and post radiotherapy in phase 3 and Phase 1 trials. We will continue our xenograft studies as it pertains to fractionated and single dose related growth delay experiments, and to ascertain the relative senescence-associated in DNA repair-associated markers that we have observed based on the manuscripts completed related to Task 1. As such we have already started some components of Task 3 and these studies will run long-term and parallel as patient biopsies are accrued for the assays.

KEY RESEACH ACCOMPLISHMENTS:

1. Determination of clonogenic radiation survival for both normal malignant prostate epithelium, showing that normal epithelium is exquisitely sensitive to radiation which may therefore explain the glandular atrophy during clinical prostate radiotherapy.
2. Apoptosis is not the dominant mode of cell death in prostate cancer cells following ionizing radiation. Clonogenic survival can be better estimated by the number of cells undergoing a tumour arrest or senescence like phenotype which may explain the long time to PSA nadir in irradiated prostate patients..
3. Molecular markers pertaining to apoptosis and molecular endpoints of apoptosis are not correlated in malignant and normal prostate cell lines.

4. DNA repair complexes can be visualized using confocal microscopy and quantitative and are dose dependant for the RAD50, RAD51, BRCA2-associated complexes. The appearance and disappearance of these foci correlate to the kinetics of DNA biochemical rejoining assays (ie. Comet assay).
5. Malignant prostate epithelium has an inherent DNA defect in terms of DNA double strand break repair, single strand break repair and base damage repair. This may be related to carcinogenesis as well as irradiation response. The defect in repair can be associated with the defect in the RAD51 nuclear foci formation, even though these cell lines exhibit high levels or RAD51 protein expression on western blot analyses. There is therefore a disconnect between these two endpoints which speaks to function rather than expression.
6. Xenografts have been received from Rotterdam and are growing in nude mice. Preliminary single dose experiments (20 Gy) show that a number of DNA repair markers can be radiation induced and this now must be correlated to the overall radiation response in terms of in vivo growth delay analyses.
7. Accrual to the phase I pilot study of preoperative radiotherapy has achieved in 8 patients so far in which biopsies are available in pre and post radiotherapy for staining of DNA-repair associated markers.
8. These same biopsies have been successfully sequenced for the p53 gene in terms of all exons (2-11) and are being correlated with immunohistochemical endpoints relating to p53 function and expression and apoptosis.

Reportable Outcomes:

Manuscripts:

Milosevic M, Toi A, Sweet J, **Bristow R,** Warde P, McLean M, Crook J, Catton C, Catton P, Gospodarowicz M. Trans-rectal oxygen measurements in prostate cancer. Clin Invest Med 23 (Supp), S19, 2000.
Parker C, Milosevic M, Toi A, Sweet J, Panzarella T, Syed A, **Bristow R**, Catton C, Catton P, Crook J, Gospodarowicz M, Maclean M, Warde P, Hill R A polarographic electrode study of tumor oxygenation in localized prostate cancer; (Conditionally accepted, Radiotherapy and Oncology, 2002)
Bromfield G, Fan R, Meng A, Kumaravel Ts, **Bristow RG**. Radiation-Induced Death Pathways in Prostate Cells: Role of Apoptotic and Non-Apoptotic Cell Death (In press, Prostate Cancer and Prostate Diseases, Fall 2002).
Fan, R., Kumaravel, TS, Bromfield G.and **Bristow, R**. "Defective rad51 nuclear localization in malignant prostate cancer. Manuscript in preparation, Fall, 2002

Abstracts and Presentations:

Bromfield, G P, **Bristow, R G**. "Radiation-Induced Cell Death Pathways in Normal and Malignant Prostate Epithelial Cells", Radiation Research Society, San Juan, Puerto Rico, April 2001.

Bromfield, G P, **Bristow, R G.** "Relative Importance of Non-Apoptotic and Apoptotic Cell Death Pathways in Irradiated Prostate Cells", CARO Meeting, Quebec City, September 2001.
Bristow R. G., Bromfield G, Fan R, Meng A, Kumaravel TS. "Radiotherapy-induced death pathways in prostate cells: Apoptosis versus permanent cell cycle arrest", 57th annual CUA Meeting, Newfoundland, June 2002.
Fan R, Kumaravel TS, Bromfield G, Jalali F, Meng A, **R. Bristow**. "DNA Repair in normal and malignant prostate epithelial tissues: implications for genetic stability and radiotherapy", ESTRO, Prague, September 2002.

Invited Lectures-R. Bristow:

"New Aspects of Radiation Oncology in Prostate Cancer", Astra-Zeneca Update on Urology, Invited Speaker, Deerhurst, Ontario, May 2001
"Molecular Biomarkers in Radiation Oncology", Target Insight DRO-CME Meeting; Invited Speaker; May 2001.
"The New Genetics and Radiation Oncology: Defining a Molecular Therapeutic Ratio" Invited Speaker, Cobalt 50th Anniversary in London, Advances in Radiation Therapy Symposium, London, Ontario, October 2001.
"Novel Biomarkers of DNA Damage Responses: Implications and Limitations for Predicting Radiotherapy Response", Invited Speaker, NIH Bio-targeting Workshop. Washington, April 2002.
"DNA Repair in normal and malignant prostate epithelial tissues: implications for genetic stability and radiotherapy", NIH Young Investigator's Meeting, NIH-Bethesda, August 2002.

Other reportable outcomes:

Ms. Gillian Bromfield obtained an MSc in Medical Biophysics (U Toronto) utilizing operating funds from this award relating to apoptosis and senescence in irradiated prostate cells. Furthermore, there is a continues development of the tissue bank relating to irradiated prostate specimens as it relates to the phase I and phase III clinical trials. Funding was applied to the Canadian Prostate Cancer Research Initiative (CPCRI) regarding HDAC inhibitors and prostate cancer senescence, and a seed grant ($ 37,500 CAN has been awarded. Although the proposed new studies are completely separate from the current US Army DOD grant, information gleaned from the US Army grant supported new hypotheses in the CPCRI grant.

Ms. Alice Meng, M.Sc. was hired as the technician for the technical aspects of the grant as outlined in the original budget. She came to our lab from the private biotechnology sector.

Dr. Rong Fan, a post doctoral fellow recruited following a previous post-doctoral fellowship at Harvard University, is supported by this US Army award. She has done extremely well in her studies relating to RAD51 and prostate cancer carcinogenesis in

radiation response. A manuscript will ensue from this work shortly in the next 3-6 months.

Conclusions:

The importance of our work suggests that we can focus on DNA repair as an important endpoint in the radiation response of prostate cancer, given that we have shown the terminal growth arrest and cells senescence are associated with clonogenic survival rather than apoptosis.

Our important results currently suggest that cell senescence is an important factor that must be further looked at in terms of pharmacologic manipulation in relation to prostate cancer cell kill following radiation. Out studies also suggest that there are defects in DNA repair the novel in malignant cell lines compared to normal epithelial cell lines, and this is being investigated with confocal immunofluorescent biology in terms of function and repair complex formation (5). Currently our data suggests that in malignant prostate cancer, the RAD51 is dysfunctional even though it is expressed at high levels, which may explain prostate cancer carcinogenesis and relative response to radiation therapy(1). These studies also suggest that anti-RAD51 therapy, such as gene therapy using alogo nucleotides or adenoviral vectors(3), may be efficacious in targeting malignant versus normal prostate cells, given that the RAD51 protein is dysfunctional in malignant cancer epithelium.

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12 August 2002

Prostate Cancer
AND PROSTATIC DISEASES

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Dear Dr Bristow

Re: Prostate Cancer and Prostatic Diseases – Manuscript 000223

Thank you for sending in your revised paper entitled "Cell Death in Irradiated Prostate Epithelia Cells: Role of Apoptotic and Clonogenic Cell Kill". I am pleased to be able to inform you that the paper has now been accepted for publication in the journal *Prostate Cancer and Prostatic Diseases* and I have today forwarded it to the publishers for typesetting. You should receive author proofs in a couple of weeks.

Thank you for taking the time to submit to this journal.

Kind regards
Yours sincerely

Roger S Kirby
Professor of Urology
Editor

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Cell Death in Irradiated Prostate Epithelial Cells:

Role of Apoptotic and Clonogenic Cell Kill

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Running Title: ***Death In Irradiated Prostate Epithelial Cells***

Key Words: prostate cancer, epithelium, senescence, apoptosis, radiosensitivity

ABSTRACT

Dose-escalated conformal radiotherapy is increasingly being used to radically treat prostate cancer with encouraging results and minimal long-term toxicity, yet little is known regarding the response of normal or malignant prostate cells to ionizing radiation (IR). To clarify the basis for cell killing during prostate cancer radiotherapy, we determined the IR-induced expression of several apoptotic- (bax, bcl-2, survivin and PARP) and G1-cell cycle checkpoint- (p53 and $p21^{WAF1/Cip1}$) related proteins, in both normal (PrEC-epithelial and PrSC-stromal) and malignant (LNCaP, DU-145 and PC-3; all epithelial) prostate cells. For these experiments, we chose doses ranging from 2 to 10Gy, to be representative of the 1.8-2Gy daily clinical fractions given during curative radiotherapy and the 8-10Gy single doses given in palliative radiotherapy. We observed that IR-induced bax and $p21^{WAF1/Cip1}$ protein expression were attenuated selectively in normal stromal and epithelial cell cultures, yet maintained their p53-dependency in malignant cell lines. For each cell culture, we also determined total apoptotic and overall radiation cell kill using a short-term nuclear morphologic assay and a long-term clonogenic survival assay, respectively. Clonogenic survival, as measured by the surviving fraction at 2Gy (SF2), ranged from 0.05 (PrEC) to 0.55 (DU-145), suggesting that malignant prostate cells are more radioresistant than normal prostate cells, for this series. IR-induced apoptotic cell kill was minimal (less than 6% cell after a dose of 10Gy at times of 24-96 hours) and was not dose-dependent. Furthermore, apoptotic kill was not correlated with either molecular apoptotic response or clonogenic cell kill. Using a flow cytometric proliferation assay with the PrSC (stromal) and DU-145 (epithelial) representative cultures, we observed that a senescent-like phenotype (SLP) emerges within a sub-population of cells post-irradiation that is non-clonogenic. Terminal growth arrest was dose-responsive at 96 hours following irradiation and associated with long-term expression of both $p21^{WAF1/Cip1}$ and $p16^{INK4a}$ genes. Future strategies for prostate radiotherapy prediction or novel treatments should additionally focus on terminal growth arrest as an important endpoint in prostate cancer therapy.

Key Words: prostate cancer, epithelium, senescence, apoptosis, radiosensitivity

INTRODUCTION

Dose-escalated (i.e. 76-80Gy) radiotherapy is an important treatment option for men with intermediate-risk prostate cancer who present with T1or T2 disease, a Gleason score greater than 6 out of 10 and serum prostatic specific antigen (PSA) values in the order of 10-20ug/ml[1]. Successful radiotherapy results in a gradual decline of the serum PSA over 12-24 months following treatment where a PSA nadir of less than 1.0ug/ml predicts for 5-year, long-term local control[2]. With 3-dimensional conformal (3D-CRT) or intensity-modulated (IMRT) radiotherapy treatment protocols, 5-year PSA-free relapse rates are approximately 75-85% and associated with minimal late toxicity (less than 5% with Radiotherapy Oncology Group (RTOG) Grade 3-4 rectal or bladder damage)[2].

Yet these same data predict that 15-25% will not achieve local control following radical radiotherapy, thought in part to be due to the intrinsic radioresistance of prostate cancer cells secondary to genetic (e.g. apoptosis, cell-cycle or DNA-repair-related gene expression) or microenvironmental factors (e.g. hypoxia or altered growth factor expression)[3]. The success of radiation therapy in prostate cancer treatment is therefore dependent on the eradication of all prostate tumor clonogens (i.e. tumor stem cells, estimated to be less than 1% of cells within a tumor), which are select tumor cells capable of unlimited proliferative potential. Failure to eradicate this particular population will result in the re-growth of the tumor following treatment[4]. Despite many advances towards understanding prostate carcinogenesis, little work has been published to date relating the mode of clonogenic cell kill following DNA damage in either normal and malignant prostate epithelial cell cultures. This information is important in defining new strategies to augment prostate cancer cell kill and understand the kinetics of cell kill following radiotherapy (in comparison to decreasing PSA value kinetics *in vivo*) and may allow optimal interpretation of post-radiotherapy biopsies as a secondary measure of local tumor control.

Apoptosis plays an important role in the death of both normal prostate and androgen-dependent malignant prostate tissue following androgen withdrawal, leading to a decrease in either glandular or tumor volume, respectively. This is supported by the observation of rapid apoptosis following castration in the rat prostate glands[5,6] (a response lacking in mice deficient in expression of the pro-apoptotic gene, bax [7]) and in patients treated with androgen-withdrawal therapy for locally advanced or metastatic prostate cancer[8]. The relationship between androgen ablation and apoptosis has led to a number of clinical studies that have attempted to determine whether local failure following radical radiotherapy is secondary to genetic factors that control radiation-induced apoptosis. However, taken together, clinico-pathologic studies that have attempted to correlate altered expression of the p53, $p21^{WAF1/CIP1}$, bax, bcl-2 and caspase apoptosis-related genes and radiocurability have been

inconclusive[9,10]. This may be due to small clinical sample size, differences in the quantitation or timing of immunohistochemical or gene expression endpoints[11,12], variable clinical treatment parameters, or perhaps due to alternate mechanisms of prostate cell death that confound analyses focused solely on apoptotic endpoints.

Recent data has supported the hypothesis that apoptosis may not be the dominant mode of cell death following radio- and chemotherapy in stromal (i.e. fibroblast) and epithelial tissues[13,14]. Indeed, it has been suggested that in these and selected tumor models, apoptosis may actually occur in a non-clonogenic population following DNA damage[15,16]. Alternatively, a permanent cell-cycle arrest or senescence-like terminal growth arrest may also be a factor in determining prostate cell death following radiotherapy[17]. Markers of senescence, such as senescence-associated β-galactosidase (SA β-gal) activity and permanently elevated levels of $p21^{WAF1/Cip1}$ and $p16^{INK4a}$ are actively under investigation as biomarkers of terminal growth arrest in human tumors[18]. Chang and co-workers [19] observed that a number of DNA-damaging agents (including ionizing radiation) could induce a senescence-like phenotype (SLP) in 11/14 cell lines tested. Other laboratories [13] and reviews have outlined a number of considerations regarding radiation-induced cell-death, including cell-type dependency in defining the dominant mode(s) of death (i.e. apoptosis, mitotic-linked death and reproductive death (SLP)/necrosis)[20]. An improved understanding of death processes has been afforded by novel flow cytometric methods to detect terminally-arrested tumor cells following drug treatment[19,20], and ascertain their relative morphology and clonogenic potential.

The purpose of the current study was to examine the mode(s) of prostate cell death *in vitro* following exposure to ionizing radiation to possibly refine clinical biomarkers for prediction of radiotherapeutic response and suggest future treatment strategies. Apoptosis, proliferation and clonogenic survival were assessed in a panel of cell lines comprised of both normal (stromal-PrSC and epithelial-PREC) and malignant (LNCaP, DU-145, PC-3; all epithelial) cultures, to determine the overall cellular response. We demonstrate that apoptosis is not the dominant mode of cell kill in this panel of cell cultures post-IR. Instead, data on selected cell lines supports the concept that long-term proliferative arrest relates to clonogenic radiation cell killing *in vitro*.

MATERIALS AND METHODS

Cell Culture

All cell cultures were incubated in vented tissue culture flasks under 5% CO_2 and 37°C culture conditions. LNCaP cells (a gift from L. Chung, University of Virginia) were maintained in T-media (Gibco-BRL) and supplemented with 10% FCS. PC-3 and DU-145 cells were purchased from ATCC and maintained as suggested in Ham's F12K, and alpha-Modified Eagles Medium respectively,

supplemented with 10% FCS and 2mM L-glutamine. PrEC (normal prostate epithelial cells) and PrSC (normal prostate stromal cells) were purchased from Clonetics and maintained per suggested protocol in PrEGM media and SCGM media, without testosterone supplementation, respectively. Both cell cultures have limited lifespan and proliferative potential in culture according to the supplier and we have consistently observed decreased growth rates following passage 5 *in vitro* for both cultures. Immortalized myc-infected Rat-1 HO15.19 fibroblast cells (a gift from Dr. L. Penn, OCI/PMH[21]) were maintained in Dulbecco's H21 media supplemented with 10% FBS. Cultures were maintained without testosterone supplementation to ensure that radiation survival studies were completed under similar conditions in normal and malignant prostate cultures and as previous experiments have determined that exogenous testosterone may not always alter apoptotic responses, clonogenic survival or IR-induced $p21^{WAF1/Cip1}$ expression and cell arrest in androgen-*sensitive* cells (J. Tsihlias, personal communication, and [22,23]). Approximate doubling times for cell cultures under these conditions were as follows: PrEC: 36-48 hours (highly variable), PrSC: 18 hours, LNCaP: 36 hours, PC-3: 24 hours, DU-145: 18 hours, Myc-expressing Rat-1HO15.19: 16 hours.

SF2Gy and Clonogenic Radiation Survival Curves

Logarithmically growing cells were rinsed with PBS/HBSS, trypsinized for 5 minutes at 37°C, and then were seeded at appropriate densities for colony formation in six-well dishes (Nunc). Asynchronous cultures were irradiated 16-20 hours post-plating to reduce the immediate effects of trypsinization and such that the multiplicity index was less that 1.1 [24]. At least two dilutions of cells in triplicate were used for each dose point for any given individual experiment. At least three independent clonogenic radiation experiments were completed for each cell line. Plated cells were either mock irradiated or irradiated with 0 to 10Gy under aerobic conditions using a ^{137}Cs irradiator at ~ 1Gy/min at room temperature. Plates were then incubated at 37°C for 7-14 days depending on cell doubling time *in vitro* and re-fed every 4-5 days before fixation and staining (methylene blue/50% methanol) of resulting colonies (aggregates of greater than fifty cells were scored as a colony). Radiation survival was calculated as the plating efficiency of treated cells divided by the plating efficiency of untreated cells and plotted as a function of dose on a semi-logarithmic plot as previously described [24]. We were unable to derive colonies at doses greater than 2Gy in the LNCaP and PrEC cell cultures due to poor plating efficiencies, and therefore only SF2 values are presented for these cultures. For a given cell culture, there was no correlation between the SF2 values and plating efficiency amongst individual experiments, although mean SF2 values were increased in cell lines which exhibited increased plating efficiency, as reported in the literature (i.e. DU-145 and PC-3) [3,21].

Western Blotting

Logarithmically-growing cells were irradiated and lysed on ice for 20 minutes with E7 lysis buffer as previously described [24]. Protein quantification was performed determined using a commercial Pierce-BCA assay kit to derive a mean concentration value based on three assays per lysate. SDS-PAGE was performed using 7-12% bis-acrylamide (29:1) gels with a 4% stacking gel run in a Novex X-cell semi-dry Mini Cell western blotting apparatus at room temperature. Each well was loaded with 20μg of total protein plus loading buffer (final concentration 1x-6% glycerol, 0.83% β-mercaptoethanol, 1.71% Tris-HCl pH 6.8, 0.002% Bromophenol Blue) after boiling for 3 minutes. Samples resolved by electrophoresis at 80-110 Volts for 1.5-2.5 hrs were transferred onto nitrocellulose overnight at 14v/4°C or for 1.5 hours at 24v/room temperature in transfer buffer (75mM glycine, 10mM Tris, 20% methanol). For selected blots, pre-hybridization staining with Ponceau S confirmed equal loading and transfer between running lanes.

To detect protein, membranes were blocked in TBST/0-10% low fat milk and then exposed to the primary antibody 2-4 hours at room temperature constant rotation. Membranes were then rinsed with TBST and exposed to the appropriate secondary antibody for 1 hour under similar conditions, rinsed again with TBST, once with 10x TBS and finally incubated in Amersham ECL chemiluminescence solution for one minute. Membranes were exposed to Hyperfilm ECL from AmershamPharmacia and analyzed by densitometry (Molecular Dynamics Computing Densitometer, ImageQuant Mac v.1.2). Primary antibodies used in these studies included: p53-mouse monoclonal (Santa Cruz Bp53-12, 1:3000); $p21^{WAF1}$-mouse monoclonal (Oncogene Ab-1, 1:3000); Bax-rabbit polyclonal (Santa Cruz N-20, 1:1000); Bcl-2-mouse monoclonal (Santa Cruz 509, 1:500); PARP-mouse monoclonal (BioMol SA-249, 1:1000), survivin-rabbit polyclonal (Alpha Diagnostics SURV11, 1:5000), and $p16^{INK4A}$ mouse monoclonal (Oncogene Ab-1, 1:1000).

Assays for Apoptotic Cell Death

Radiation-induced apoptosis was quantified on the basis of distinct nuclear morphology and associated apoptotic bodies based on a previously standardized immunofluorescence protocol (Hoechst 33342 staining)[24]. For the morphology assay, logarithmically growing cells were re-plated at appropriate densities in triplicate and mock/irradiated with 0, 2, 10 or 20Gy. These were scored for apoptotic morphology (i.e. apoptotic bodies and nuclear condensation-see sample in Figure 2a) at periods of 24 to 96 hours following irradiation. Total adherent and floating cells in each culture were fixed and stained in 4% formalin-PBS/10μM Hoechst 33342 DNA-specific dye for 30 minutes at RT. Cell counts to evaluate any cell loss/lysis into culture media were also performed at each time-point.

All experiments utilized Rat-1 HO15.19 cell line as positive control for gamma-irradiation induced apoptosis (L. Penn, personal communication and Lee et al.[25]).

Radiation Survival in Proliferating and Non-proliferating Irradiated Cultures: The CFDA-SE Flow Cytometry Proliferation Assay

To determine if permanent arrest is associated with decreased clonogenic survival, a modification of the protocol by Chang et al.[19] utilized the CFDA-SE (CFSE) fluorescent dye[20,26]. The CFSE compound (Molecular Probes, C-1157) is distributed throughout the cellular membranes and is divided evenly amongst subsequent progeny based on division of equal volumes of membrane at cell division. Multiple rounds of cell division are therefore represented by a corresponding decrease in total membrane fluorescence within a proliferating population, which can be detected by flow cytometry. Analyzing cell populations for relative fluorescence (FL1[CFSE] parameter; increased in non-proliferating cultures) and increasing side-scatter (SSC parameter; due to increased granularity associated with senescent cells) allows for flow cytometric analysis of senescent-like populations post-treatment.

Sub-confluent flasks of cells were trypsinized, collected and centrifuged into a pellet and $5x10^6$ cells were re-suspended in 1mL serum-free media plus 1uL of stock solution (5M CFSE in DMSO) at 37°C for 10 minutes with occasional inversion. Ice-cold RPMI 1640 + 10% FBS was then added prior to a subsequent cell centrifugation, and finally the cells were re-suspended in PBS. The cells were further washed twice in PBS, re-plated at low density (approximately 10% confluence) into multiple, $175cm^2$ Falcon flasks for next-day irradiation (0 to 10Gy). As a control, all cells (floating and adherent) from one untreated flask were harvested one day post-plating and analyzed with flow cytometry to find baseline fluorescence (FL1[CFSE]) intensity. Remaining cultures were followed until day 5 when all cells (floating and adherent) were harvested, analyzed and sorted by FACS into "non-proliferating" ($FL1[CFSE]^{hi}SSC^{hi}$), and "proliferating" (i.e. all cells other than $FL1[CFSE]^{hi}SSC^{hi}$) populations to determine clonogenic potential within each population. The 5 day time point was initially chosen as it represents the point at which surviving cells would begin to declare their colony-forming ability[3]. Sorted populations of DU-145 and PrSC were used to derive colonies in each sub-group as examples of epithelial and stromal (i.e. fibroblast-like) models. Pre-sort samples were analyzed on a BDIS FACScan analyzer and samples sorted using either a Becton Dickonson Immunocytometry system FACStarPLUS or BDIS FACS Vantage system. BDIS CELLQuest Software v3.3 was used for both sorting and analysis. Cell lysates from adherent and floating cells in parallel cultures treated similarly (stained, irradiated with 0, 2 and 10Gy) were also harvested on days 5-9, and analyzed for expression of the $p16^{INK4a}$ and $p21^{WAF1}$ genes. Cultures were also stained for

senescence-associated β-galactosidase (SA β-gal) using the method of Chang et al.[19], as a complementary biomarker of senescence-like death.

RESULTS

Gene Expression of Apoptosis-related Genes Within Irradiated Prostate Cell Cultures

As different laboratories may contain variants of original cell stocks, we initially determined the p53 status of the malignant prostate epithelial cell lines using full-length DNA sequencing of exons 1-11 of the p53 gene. Consistent with previous reports, LNCaP cells were found to express two wild type (WT) alleles, whereas the PC-3 cells were devoid of p53 protein expression due to chromosome 17p hemizygosity and a mutation in the remaining allele at codon 138 which results in a premature stop codon at position 169. The DU-145 cells express mutant (MT) p53 protein due to mutations at codons 223 and 274. We observed that the level of bax protein expression is p53-dependent following IR, given the increased expression in the WTp53-expressing LNCaP cells at 24 hours following 10Gy. This molecular response was attenuated, or absent, in the remaining PC-3 and DU-145 malignant epithelial cell lines, which have altered p53 protein expression. Of note, despite the western blots shown in Figure 1b, bax expression is detectable in DU-145 cells, albeit at a very low level. The response was also attenuated within the normal epithelial and stromal cultures (see Figure 1a, b, Table 1). Bcl-2 protein levels were low, yet detectable, using our antibody and remained unchanged in all cell cultures following 10Gy for periods up to 24 hours following radiation (see Figure 1c, d). In other experiments in our laboratory, the relative levels of bax and bcl-2 protein have been confirmed at the mRNA level by RNA protection analyses (Fan R. and Bristow R.G., manuscript in preparation). The IAP-related protein, survivin, has been suggested to be a prognostic indicator in a variety of cancers, presumably due to its influence on cellular apoptosis and G2 phase cell cycle control[27] and reported differential upregulation in malignant tissues, relative to normal tissues[28]. *In vitro*, endogenous survivin levels were observed to be detectable in all of the cell cultures within our series (normal and malignant), and were invariant following irradiation (Table 1) except for a slight (1.6-fold) increase in PrSC. Subsequent experiments have revealed lower levels of survivin in PrEC cultures than in their stromal and malignant counterparts.

Terminal apoptotic transduction involves altered expression and cleavage of the caspase family of proteins and the PARP protein[29,30]. Other laboratories have reported that caspase 1 and 3 protein expression can be deficient in some of our malignant cell lines[31]. In our experiments, analysis for PARP cleavage revealed that, although we detected an increase in the characteristic 89kD apoptotic-related PARP protein fragment in our highly-apoptotic Rat-1 HO15.19 cell line, there was no such change in any of the five prostate cell cultures at 24 hours following a dose of 10Gy (data not shown).

Our results (summarized in Table 1) suggest that gene and protein expression related to terminal apoptosis-defining events following IR is not consistent with observations made for other cells (e.g. lymphocytes or thymocytes) which are classically more susceptible to radiation-induced apoptosis; however we recognize that the molecular apoptotic and morphologic (see below) response of our cell panel may be variable dependent on type of cell stress and drug treatment.

Minimal Evidence of Apoptotic Cell Death in Irradiated Prostate Cultures

We next determined the level of radiation-induced apoptosis following various doses (2-20Gy) and time points (0-96 hours post-IR) using a distinct nuclear morphology assay (see Figure 2a) as previously described[24]. A dose of 10Gy has been shown to decrease clonogenic survival by 3 logs or more, in other malignant cell lines. The highly-apoptotic adherent Rat-1 HO15.19 cell line formed a positive control for IR-induced apoptosis in these experiments and irradiated cultures were observed to contain decreasing numbers of cells over 24-96 hours consistent with a rapid induction of cell death (data not shown). The level of apoptosis in Rat-1 HO15.19 control following 20Gy became difficult to quantify at 24 hours due to a large amount of cellular debris and may be underestimated as presented in Figure 2b. The apoptotic response of our prostate cell panel at times of 24 to 96 following 10Gy as determined by morphology is presented in Figure 2b, and reveals that neither normal or malignant prostate cells undergo high levels of apoptosis at any time point up to 96 hours (a time at which the earliest colonies indicative of clonogenic survival can be detected). Additionally, careful total cell counts of adherent and floating cells within all the irradiated cultures suggested that there was no decrease at any time point up to 96 hours in total cell number, which ruled out underestimating apoptotic responses (data not shown). Moreover, in contrast to the Rat-1 HO15.19 Myc-expressing control, there was no evidence for a dose-dependent increase in apoptosis in our panel of cultures (2Gy range=0-1%, 10Gy range=0-6%, 20Gy range=0-3%; see Figure 2c).

We observed a trend towards increased levels of apoptosis among the malignant cell lines as compared to the normal cell cultures, although these relative differences were not consistent. The data presented using the *in vitro* morphology assay was also supported by the absence of an apoptotic sub-G1[29] peak within DNA histograms of irradiated PC-3, DU-145 and LNCaP cells using flow cytometry, the lack of apoptotic morphology post-IR of the same cells as analyzed by the COMET DNA-damage assay[32], and minimal TUNEL staining of the PC-3 cell line either *in vitro* or *in vivo* (growing as a xenograft i.m., in a nude mouse host) following irradiation (data not shown). These results suggest that cellular apoptosis is not a major mechanism of IR-induced prostate cell death under the culture and treatment conditions used in this study.

Molecular Analysis of Checkpoint Control in Prostate Cells

The p53 status in normal and malignant cells can be functionally related to either apoptosis or a G1 and G2 cell cycle arrest or checkpoint, and dependent on cell type, level of DNA damage or cell stressor[33,34]. We therefore confirmed the presence or absence of a molecular p53-dependent G1 checkpoint in the normal and malignant prostate cells by determining the IR-induced upregulation of the cdk-inhibitory protein, $p21^{WAF1/Cip1}$. Following IR-induced DNA damage, the p53 protein is stabilized post-transcriptionally, by alternate phosphorylation of its amino terminus at serine residues 15 and 20 through both direct and indirect actions of the ATM protein. Stabilized p53 protein can then lead to a transcriptional upregulation of the $p21^{WAF1/Cip1}$ protein, which inhibits the G1 cyclin-cdk kinase complexes, and results in a G1 arrest secondary to hypo-phosphorylation of the pRB (retinoblastoma) protein. An increased level of p53 protein was observed following irradiation in all WTp53-expressing prostate cells and peaked at 2-6 hours following IR-treatment (Figure 3). As expected, we observed a lack of p53 protein expression in irradiated null-p53 PC-3 cells and elevated endogenous levels of p53 protein in the MTp53-expressing DU-145 cells (consistent with a longer half-life for the MTp53 protein; Figure 3b), which were invariant post-IR. The PrEC and PrSC normal cultures both showed similar stabilization of the p53 protein relative to α-tubulin levels following irradiation. However, in the PrSC cells, the $p21^{WAF1/Cip1}$ levels were upregulated and sustained at 24 hours; in the PrEC cells, the response was relatively attenuated in level and was duration reaching almost pre-irradiation levels at 24 hours (Figure 3a). We failed to observe an increase in $p21^{WAF1/Cip1}$ expression in the MTp53-expressing and null-p53 cell lines (DU-145 and PC-3 respectively)[35], however the WTp53-expressing LNCaP cell line did show a strong IR-induced upregulation of $p21^{WAF1/Cip1}$. The p53 and $p21^{WAF1/Cip1}$ protein expression results were correlated to relative mRNA levels under similar culture conditions using RNA protection analyses in separate experiments (Fan R. and Bristow R.G., manuscript in preparation). These data support that p53 can induce a molecular G1 checkpoint in both normal and malignant prostate epithelium, but highlights previous observations that, in certain normal epithelial cultures, $p21^{WAF1/Cip1}$ expression may be attenuated relative to stromal cultures[36] in a tissue-specific manner. This may relate to relative control of cell-cycle related checkpoint and carcinogenesis in these two tissues[37].

SF2Gy and Clonogenic Survival for Normal and Malignant Prostate Cultures

Colony-formation after DNA damage measures the long-term survival of cells that are capable of unlimited proliferation and summarizes all types of IR-induced modes of cell death including apoptosis, mitotic-linked death (death after 2-3 aborted divisions followed by apoptosis or necrosis) and permanent growth arrest leading to necrosis[233]. Consistent with selected reports[38,39] full

clonogenic survival curves could not be generated for our LNCaP cell line, due to poor plating efficiencies which made determination of colony-formation at doses greater than 2Gy difficult. We encountered similar difficulties with PrEC normal epithelial cultures, which also had a poor plating efficiency (0.1-1%). Nonetheless, for all cell lines we were able to generate radiation survival data after a low, clinically relevant dose of 2Gy (SF2), which approximates the daily fraction of radiation within curative radiation protocols. Full clonogenic survival curves following doses up to 10Gy (a dose approximating a single-fraction palliative treatment) were derived for the PC-3, DU-145, PrSC and Rat-1 HO15.19 cell cultures and the results are plotted in Figure 4a. The SF2 values for all cell cultures are shown in Figure 4b. The normal stromal and epithelial cultures were the most radiosensitive based on SF2 values, even though they had the lowest levels of IR-induced apoptosis at similar doses. Furthermore, the apoptotic kill response in the DU-145 and Rat-1 HO Myc cell lines was quite disparate, despite similar clonogenic survival (see Figure 2b,c). We observed that the DU-145 and PC-3 cell lines with altered p53 status were more radioresistant than the WTp53-expressing LNCaP cell line. However, defined experiments with prostate cell lines that are isogenic save for p53 status are required before concluding that p53 status correlates with radiosensitivity in prostate cancer cells. In summary, we have observed that the overall level of apoptosis was not correlated to the overall level of clonogenic cell survival in our panel of cell lines. Plotting the relative cell kill following 2Gy and 10Gy based on the two endpoints in Figure 4c illustrates the discrepancy between the results of two assays.

Permanent Arrest in Irradiated Prostate Cells

Given that apoptosis was not a dominant mechanism for clonogenic cell kill, we next investigated the contribution of terminal growth arrest associated with senescence-associated markers to clonogenic survival using both a representative stromal culture (PrSC cells), and a representative epithelial culture (DU-145 cells). The choice of these two cultures was predicated on the need for cell lines which would readily form colonies following flow cytometric sorting procedures at 2 and 10Gy and to maximize the tissue-specific and genetic differences relating to propensity for senescence, clonogenic cell kill and G1 checkpoint control.

Using a flow cytometric assay that simultaneously determines relative levels of FL1-CFSE fluorescence (i.e. proliferation) and SSC-parameter (cell granularity), we determined that up to 87% of the PrSC population was non-proliferating with an associated increased granularity ($FL1[CFSE]^{hi}SSC^{hi}$) at 5 days, following a dose 10Gy of radiation. In similarly-plated cultures, only 14% and 8% showed the same cytometric profile in 2Gy-treated or control cultures; these relative proportions being consistent over 2-3 representative experiments (Figures 5a, c). By comparison, only

32% of DU-145 cells had a senescent-like cytometric profile, although upon closer inspection, the data suggest that the vast majority (i.e. greater than 70%) of DU-145 cells were actually non-proliferating, but that these cells were inconsistently associated with increased granularity when compared to stromal PrSC cells (compare CFSE-fluorescence axis in both cell lines following 10Gy in Figure 5). The FL1[CFSE]^{hi}SSC^{hi} cytometric profile was determined for 5% of DU-145 cells following 2Gy and 3% in the untreated DU-145 population (Figure 5a, b; note that cells not growth-arrested following 2Gy or in non-irradiated cultures may have undergone multiple rounds of division by this point differentially increasing the total "proliferating" population). We then sorted and plated cells from the senescent-like, non-proliferating population relative to the remaining cells and observed that relative colony forming ability (plating efficiency) was decreased in the FL1[CFSE]^{hi}SSC^{hi} ("non-proliferating"), SLP population in a dose responsive-manner (see Table 2).

In normal fibroblasts (i.e. stromal cells), cells undergo decreasing proliferative potential with increasing passage *in vitro*, until finally undergoing senescence associated with increased granularity, positive SA β-gal staining and upregulation of the p21$^{WAF1/Cip1}$ and p16^{INK4a} proteins. Similar changes occur in normal fibroblast cultures when exposed to IR and has been referred to as a "premature IR-induced senescence"[40-42]. Although we observed greater SA β-gal staining intensity in an increased number of PrSC stromal cells following a dose of 10Gy (see Figure 6a), we found the endpoint to be highly variable across all cell lines and difficult to quantitate in epithelial cells (further data not shown; noted by others[43]). Whether upregulation of one, or both genes, are absolutely required for senescence and whether the process is p53-dependent in all cell types remains controversial.

In order to determine whether similar gene expression changes occurred in PrSC and DU-145 cells, we performed western blot analyses of cell populations obtained in parallel with flow cytometric experiments at day 5. These analyses showed high, IR-invariant levels of p16^{INK4a} protein in both PrSC and DU-145 cultures and a dose-dependent increase in p21$^{WAF1/Cip1}$ levels in PrSC cells only (Figure 6b; confirmed using densitometry). We also analyzed p16^{INK4a} expression in all of the cell cultures at 24 hours following 10Gy. In this case, levels of p16^{INK4a} were either invariant (PrEC, LNCaP, DU-145) or undetectable (PrSC, PC-3) as confirmed by relative densitometry (data not shown). Long-term (up to 9 days) analysis of p16^{INK4a} expression post-10Gy irradiation DU-145 and PrSC demonstrated increasing expression with time (data not shown), suggesting a time-dependent, thought not dose-dependent, expression of p16^{INK4a}. We conclude that cells within a terminally arrested population that have increased cellular granularity (SLP) are incapable of forming colonies and that the sub-population

of proliferating cells without associated increased granularity define the colony-forming potential of the entire irradiated culture.

DISCUSSION

This is the first report, to our knowledge, documenting the relative role of apoptosis and terminal growth arrest as factors in determining overall clonogenic radiation cell survival for a panel of normal and malignant prostate cell cultures within the same laboratory setting. Using clinically relevant radiation doses, we observed a strong molecular apoptotic response in certain cell lines (bax and p53 upregulation in LNCaP cells, post-IR), yet this response did not correlate with quantitative determinations of apoptosis using morphology endpoints. The apoptotic response was not dose-responsive and did not correlate with final clonogenic cell kill. Further support for our data are the observations that manipulation of the ceramide-sphingomyelin and bcl-2 associated apoptotic pathways, or androgen ablation, can increase the apoptotic responses of prostate cells without altering final clonogenic radiation survival (Shim K. and Bristow R.G., unpublished observation and [22,23],[44]). Many groups have indicated that apoptosis may be the primary mode of death following gamma-irradiation only in specific cell types such as hematopoetic or lymphocytic cells, but not in stromal- or epithelial-derived tissues[16,44-47]. Furthermore, many attempts to alter apoptotic indices in epithelial- and stromal-derived tissues have failed to affect clonogenic survival. This suggests that although the apoptotic pathway is intact in malignant prostate cells, other death mechanisms may over-ride this response following irradiation.

It has been suggested that epithelial cell G1 checkpoint may be abrogated or less efficient than that of its stromal counterpart, despite wild-type p53 status. Girinski et al. [36] found an abrogated $p21^{WAF1/Cip1}$ induction and G1 checkpoint in a panel of normal prostate epithelial cells as compared to a panel of normal prostate stromal cells following ionizing radiation. Meyer et al. [48] reported similar findings for mammary epithelial versus stromal tissues. Within the same group, Romonov et al. [49] observed an abrogated response to replicative crisis in the mammary epithelial cells suggesting a global defect in epithelial response to stress in comparison to its stromal counterpart. Our own experiments illustrated that, even for similar levels of p53 induction/stabilization, the $p21^{WAF1/Cip1}$ response in PrEC is greatly attenuated in comparison to its stromal counterpart. While the p53/$p21^{WAF1/Cip1}$ responses for our panel of malignant cell lines were as previously reported, the LNCaP cell line's strong $p21^{WAF1/Cip1}$ induction is still surprising in light of its epithelial nature. Girinski et al. [36] suggested that epithelial cell response might be partially dependant on interactions with surrounding stromal tissues [37,50,51]. LNCaP cells may have altered characteristics such that it does not require this interaction for a strong $p21^{WAF1/Cip1}$ induction following irradiation. Furthermore, studying G1 arrest alone may not be

sufficient, since cells lacking a functional G1 checkpoint can exhibit a G2 delay that may be linked to DNA repair[52] and cellular survival following radiation (reviewed[53]). Indeed, although our results suggest increased survival for cells which lack the G1 checkpoint, defined studies in isogenic tissue culture or solid tumor models which differ in p53 status and specific G1, S and G2 checkpoint control experiments are required to prove this hypothesis, and are part of a current research program[11].

The success or failure of radical radiotherapy depends on the daily proportionate killing of tumor clonogens, which make up less than 1% of the total population of cells within a tumor. For example, it has been estimated that each gram of tumor contains approximately 1×10^9 tumor cells, with approximately less than 1×10^7 cells having clonogenic capacity. To date, no formal test or data exists for the pre-treatment determination of the number of clonogens existing prior to prostate radiotherapy. One can estimate that for a radiotherapy protocol using 35-40 fractions of 2Gy, the clonogenic SF2 value should be less than 0.60 (i.e. death after 35 treatments = $0.6^{35} = 1.7 \times 10^{-8}$) to cure a 1 gram tumor, assuming equal killing per radiotherapy fraction[4]. Our data suggests that the SF2 *in vitro* is less than 0.6 for the cell lines tested. However, the effective SF2 may be higher *in vivo*, due to cell-cell interactions, hypoxia, altered gene expression or cell cycle phase during irradiation[3] and the relative importance of these factors remain to be in radiotherapy cohorts. Our observed *in vitro* radiosensitivity of normal epithelial and stromal cultures *in vitro* may explain the observation of glandular atrophy, fibrosis and decreased glandular function observed in prostate glands following irradiation. Exquisite sensitivity of normal epithelial cells may also explain why final post-therapy nadir PSA values in patients who achieve local control are lower than the PSA values in men without a diagnosis of prostate cancer, reflecting residual normal gland function after IR-induced cell kill[54].

In our experiments, relative clonogenic cell kill was approximated by quantitative endpoints of a dose-responsive terminal growth arrest, in which certain cells acquired a senescent-like phenotype. Terminal growth arrest, rather than apoptosis, may begin to explain the slow kinetics of decreasing PSA values following radiotherapy over 12-16 months following treatment[2]. Recent experiments by Pollack and colleagues[23,55] are consistent with our data, as the supra-additive radioresponses observed in LNCaP and Dunning rat R3327-G tumor models following combined androgen withdrawal and fractionated radiation (mimicking clinical stage T3-T4 prostate cancer treatment protocols which increase overall patient survival)[56], was secondary to factors which determined post-treatment cellular growth arrest rather than apoptosis.

If a surrogate measure of radiation sensitivity was developed to use prior to, or early during, the course of radiotherapy, specific measures could be used to increase radiocurability or abort radiotherapy altogether in favor of radical prostatectomy (if medically feasible). Crook et al. [57] found

that markers of proliferation (PCNA, MIB-1) in post- radiotherapy biopsies from 498 men were an independent indicator of treatment failure, but indeterminate biopsies do occur which complicate interpretation as to whether viable clonogenic cells remain 2 to 2.5 years following radical radiotherapy. Our data suggests that other surrogate predictive factors might include molecular or cellular senescence or cell cycle arrest factors, such as the cdk inhibitors, $p21^{WAF}$ or $p16^{INK4a}$ that are activated by DNA damage and lead to altered proliferation and terminal growth arrest.

At present, no predictive test can determine which cells in the tumor are prostate clonogens rather than non-clonogens, however we believe that terminal growth arrest should be further investigated as a major mechanism of cell death in addition to apoptosis, in protocols that utilize radiotherapy. If proven important in prostate cancer, the former mechanism of cell death might be augmented *in vivo* using radiotherapy in conjunction with inhibitors of prostate cancer cell proliferation such as retinoic acid, antisense to cdk inhibitors, inhibitors of telomerase, or inhibitors of histone deactylase (HDAC)[58,59]. Indeed these agents are currently being prospectively tested as single agents or in combination with chemotherapy in Phase I/II trials; our results suggest further studies of the efficacy of these agents in combination with radiation as a novel prostate treatment strategy.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. L. Chung donating our LNCaP cell line, and Dr. L. Penn and E. Soucie for the Rat-1 HO15.19 cell line. Special thanks to S. Al Rashid, Claude Cantin, and F. Jalali for help in selected assays and guidance. These studies were supported by the National Cancer Institute of Canada, the Princess Margaret Hospital Foundation and the U.S. Army DOD Prostate Research Program.

TABLES

Table 1: Apoptosis- and cell cycle-related gene expression in prostate cell cultures 24 hours post-10Gy irradiation, as compared to mock-irradiated controls

Normal	**p53**	**p21**	**bax**	**bcl-2**	**survivin**
PrEC	↑ ↑	↑	↔	↔	↔
PrSC	↑ ↑	↑ ↑	↔	↔	↔
Malignant					
LNCaP	↑ ↑	↑ ↑	↑ ↑	↔	↔
PC-3	n-d*	low‡	↔	↔	↔
DU-145	↔	n-d	n-d	n-d	↔

*n-d= non-detectable; ‡ low=barely detectable, no change post-XRT; ↔ less than 2-fold change; ↑ greater than 2-fold increase; ↑ ↑ greater than 4-fold increase (quantified using relative densitometry).

Table 2. Relative colony forming ability within FL1[CFSE]^{hi}SSC^{hi} (non-proliferating) and proliferating populations, as sorted by flow cytometry at day 5 post-irradiation.

Cell Type	Dose (Gy)	# of Cells Plated	Plating Efficiency (proliferating)	Plating Efficiency (Non-proliferating)
PrSC	0	100	0.33±*0.34**	0.03±*0.02*
	2	1000	0.06±*0.03*	0.01±*0.01*
	10	1000	0.00±*0.00*	0.00±*0.00*
DU-145	0	100	0.41±*0.35*	0.15±*0.14*
	2	1000	0.22±*0.17*	0.01±*0.01*
	10	1000	0.01±*0.00*	0.00±*0.00*

*mean values and associated S.E.M.

FIGURE LEGENDS

Figure 1. Western blot analyses for apoptotic-related protein expression pre- and post-irradiation, in a panel of normal and malignant prostate cell cultures: (a) bax protein expression in normal prostate epithelial (PrEC) and stromal (PrSC) cell cultures; (b) bax protein expression in malignant prostate cell cultures (Rat-1 HO15.19 cell-lysate shown as positive control); (c) bcl-2 protein expression in normal prostate cell cultures; (d) bcl-2 protein expression in malignant prostate cell cultures (human bcl-2 transfected Rat-1 fibroblast lysate shown as positive control).

Figure 2. IR-induced apoptosis in normal and malignant cell lines: (a) Nuclear morphology of selected cell populations stained with Hoechst 33342 at 48 hours following 10Gy showing evidence of apoptotic bodies and chromatin condensation in cells denoted with white arrows: left panel, irradiated LNCaP cells; right panel, irradiated RAT-1 HO15.19 positive control cells (magnification 1000x); (b) Time course of IR-induced apoptosis following a dose of 10Gy (each bar represents the mean and S.E.M.); (c) Dose-dependence of IR-induced apoptosis assayed at 48 hours (error bars omitted for clarity in 3D plot).

Figure 3. Western blot analysis of p53 and $p21^{WAF1/Cip1}$ protein expression in (a) normal, and (b) malignant prostate cultures following 10Gy irradiation.

Figure 4. Clonogenic survival data for normal and malignant prostate cultures: (a) Full clonogenic radiation survival curves for PrSC, DU-145 and PC-3 and highly-apoptotic Rat-1 HO15.19 cell cultures (each point represents a geometric mean of 3-5 independent experiments plotted with associated geometric S.E.M.);
(b) Surviving fraction following 2Gy (SF2 values) with associated S.E.M. of normal and malignant prostate cell cultures; (c) Direct comparison of mean quantitative data for apoptotic and clonogenic kill following either 2Gy (upper panel) or 10Gy (lower panel).

Figure 5: Frequency histogram of FL1 fluorescence parameter (i.e. CFSE vital dye fluorescence) for (a) PrSC (left) and DU-145 (right) at 5 days post-irradiation following doses of 0Gy (control, mock-irradiated), 2Gy and 10Gy. Shown is a representative experiment with total cell population analyzed for each dose; (b) represents 2 dimensional plots of FL1[CFSE] fluorescence intensity versus SSC (side scatter-granularity) for total cell numbers in mock irradiated and irradiated flasks of cells (see text for details) for PrSC (top) and DU-145 (bottom). Note increasing $FL1[CFSE]^{hi}$ SSC^{hi} fractional sub-population (representing a senescent-like phenotype) with increasing dose; similar cytometric data was observed in all replicate experiments. These populations as shown were sorted using flow cytometry to determine relative colony forming ability as tabulated in Table 2.

Figure 6. Senescent biomarkers in prostate cell populations: (a) Example of negative (left) and positive (right) SA β-gal staining in non-irradiated and irradiated PrSC cultures respectively, at 120 hours following a dose of 10Gy: (b) western blot analysis of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ protein expression in entire PrSC and DU-145 cultures at day 5 following irradiation.

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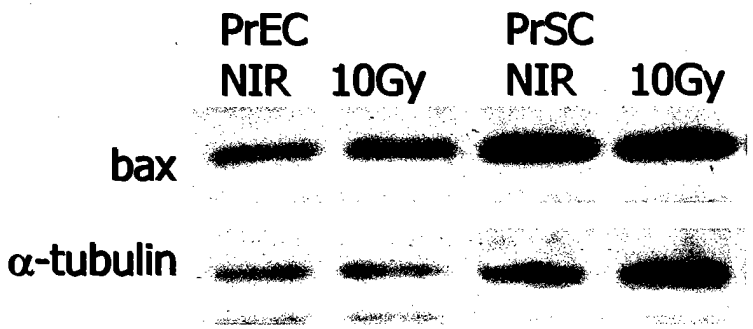
(a)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PrSC
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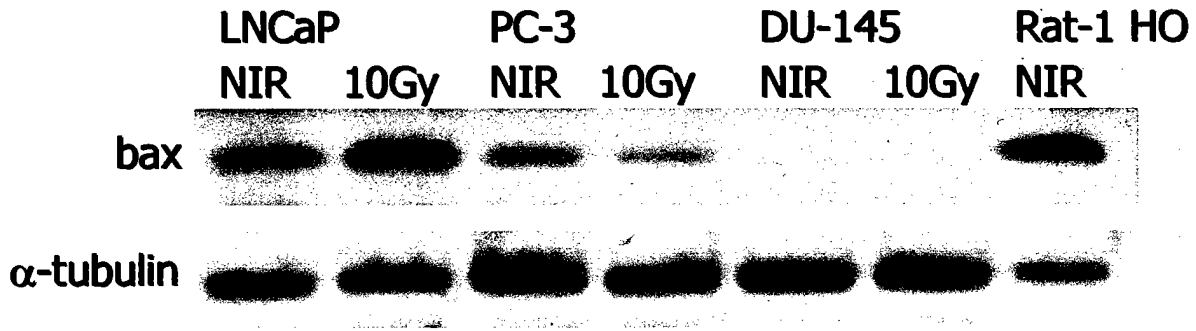
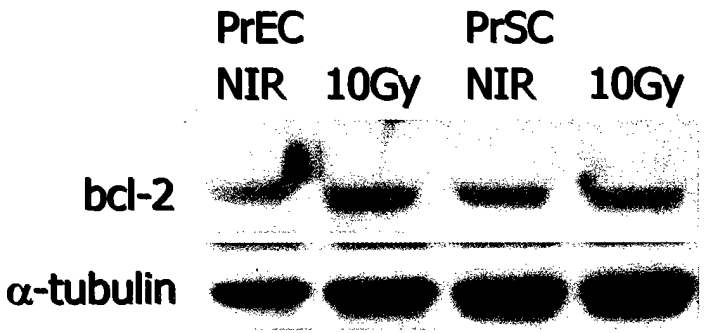
10Gy
NIR
10Gy
bax
α-tubulin

(b)


LNCaP
PC-3
DU-145
Rat-1 HO
NIR
10Gy
NIR
10Gy
NIR
10Gy
NIR
bax
α-tubulin

(c)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NIR
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bcl-2
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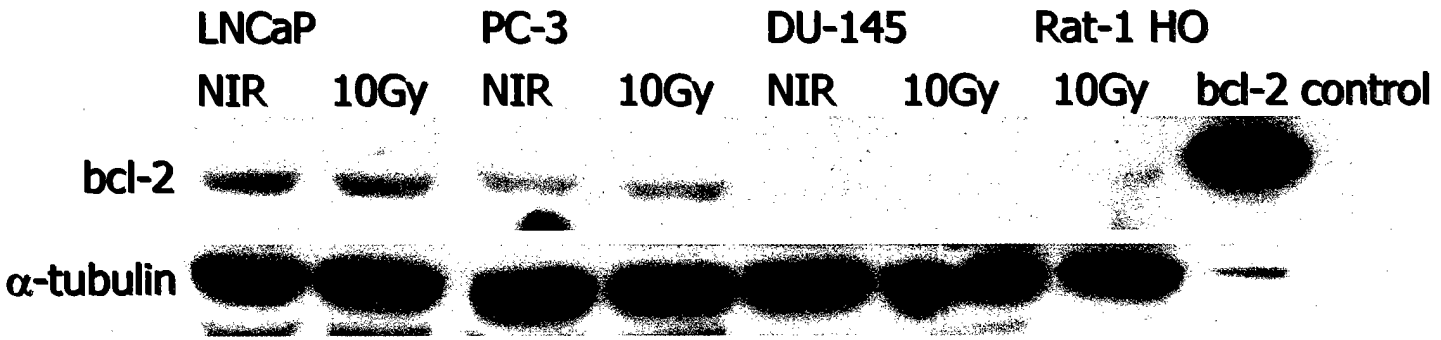
(d)


LNCaP
PC-3
DU-145
Rat-1 HO
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10Gy
NIR
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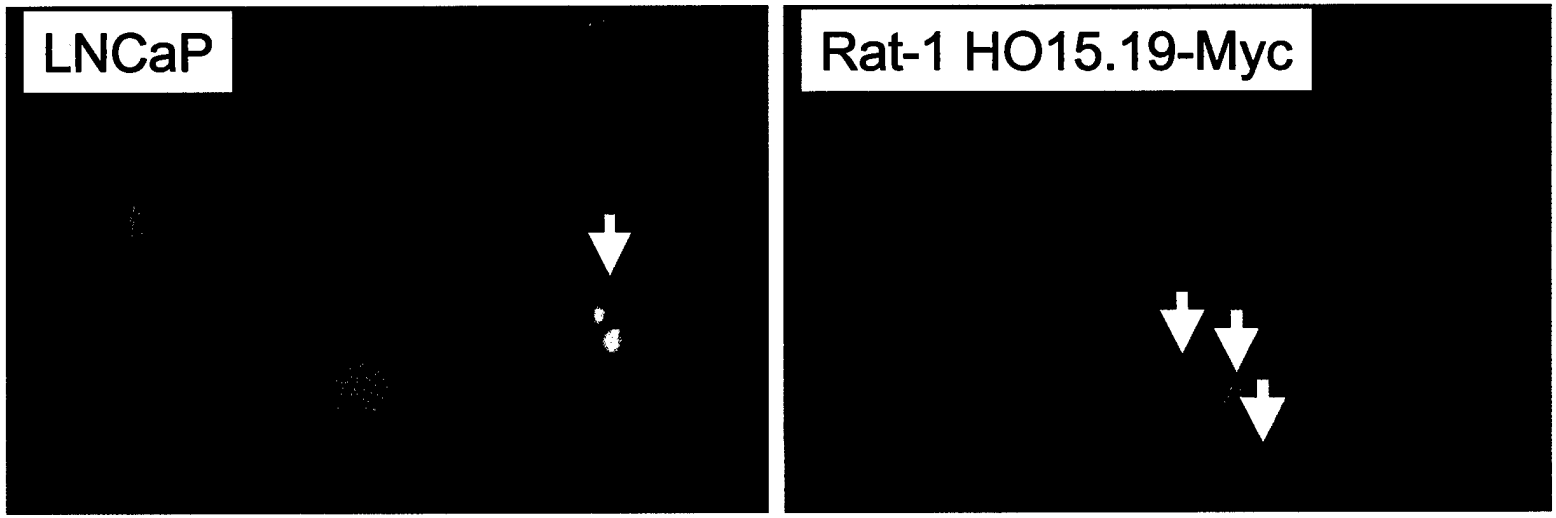
NIR
10Gy
10Gy
bcl-2 control
bcl-2
α-tubulin

(a)



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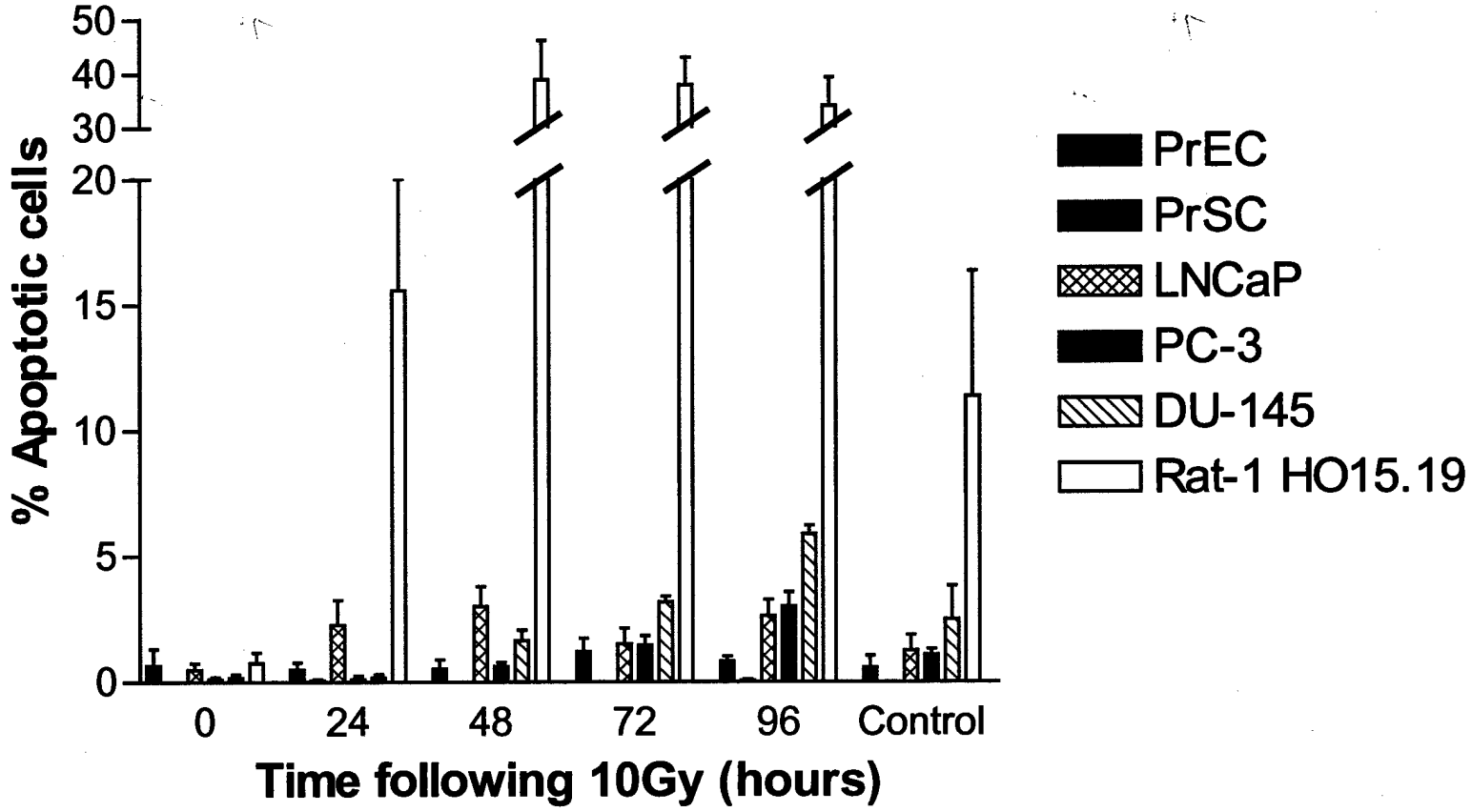
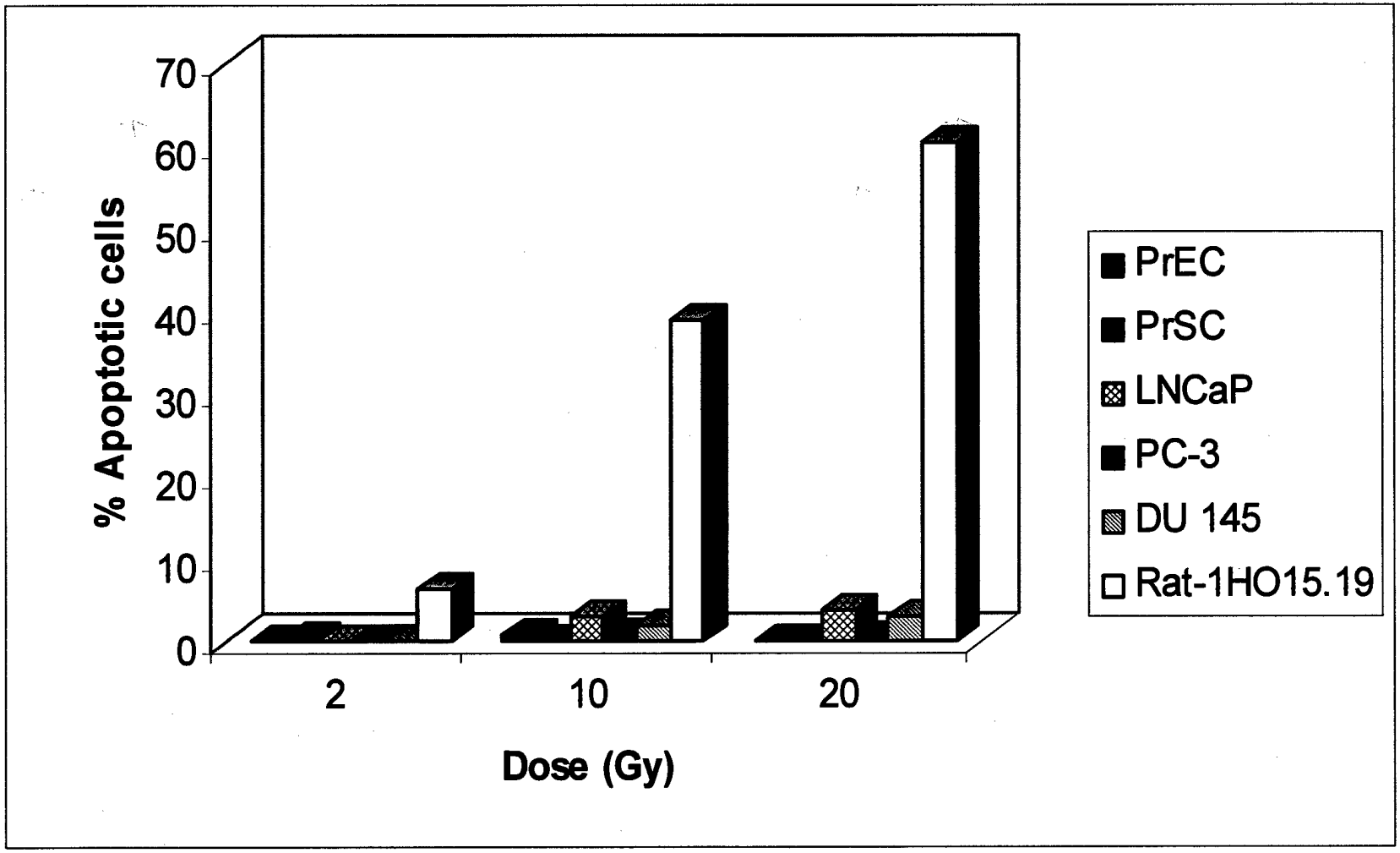
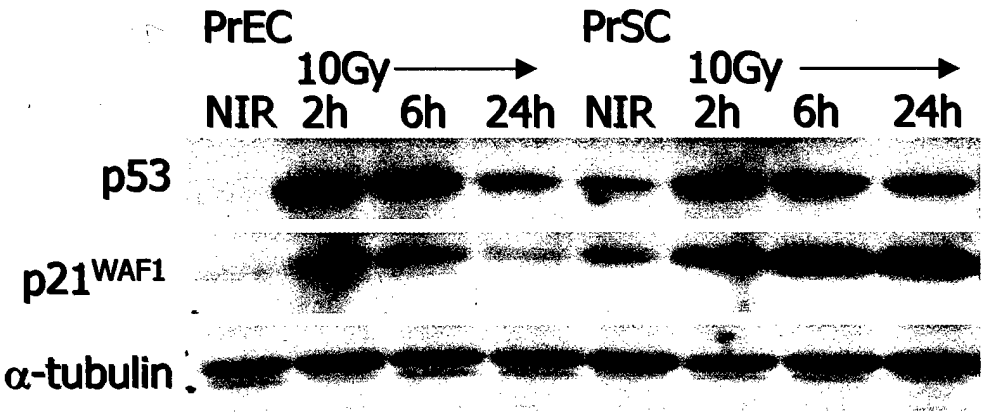

50
40
30
20
15
10
5
0
% Apoptotic cells
0
24
48
72
96
Control
Time following 10Gy (hours)
PrEC
PrSC
LNCaP
PC-3
DU-145
Rat-1 HO15.19

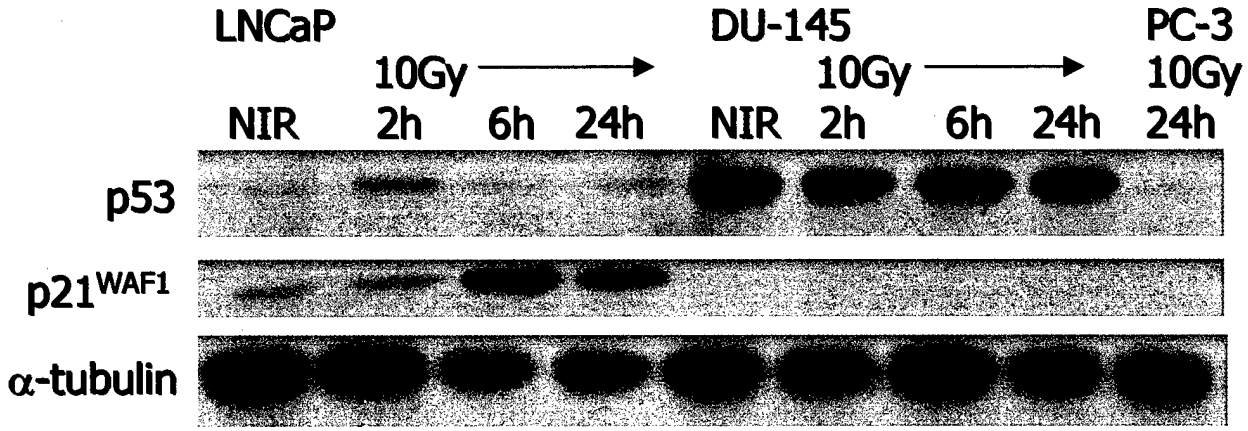
(c)


70
60
50
40
30
20
10
0
% Apoptotic cells
2
10
20
Dose (Gy)
PrEC
PrSC
LNCaP
PC-3
DU 145
Rat-1HO15.19

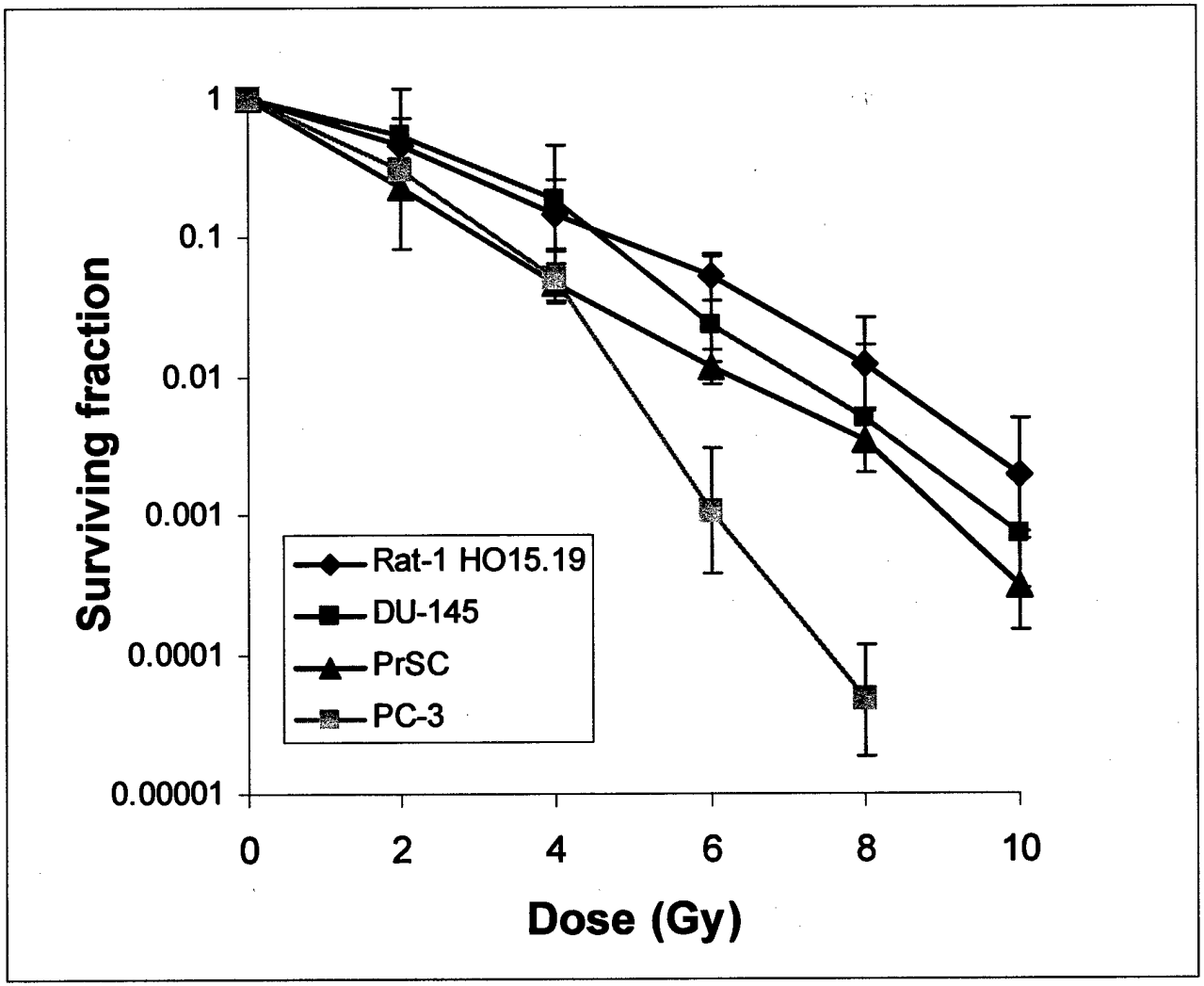
(a)


PrEC
PrSC
10Gy
10Gy
NIR 2h 6h 24h NIR 2h 6h 24h
p53
p21WAF1
α-tubulin

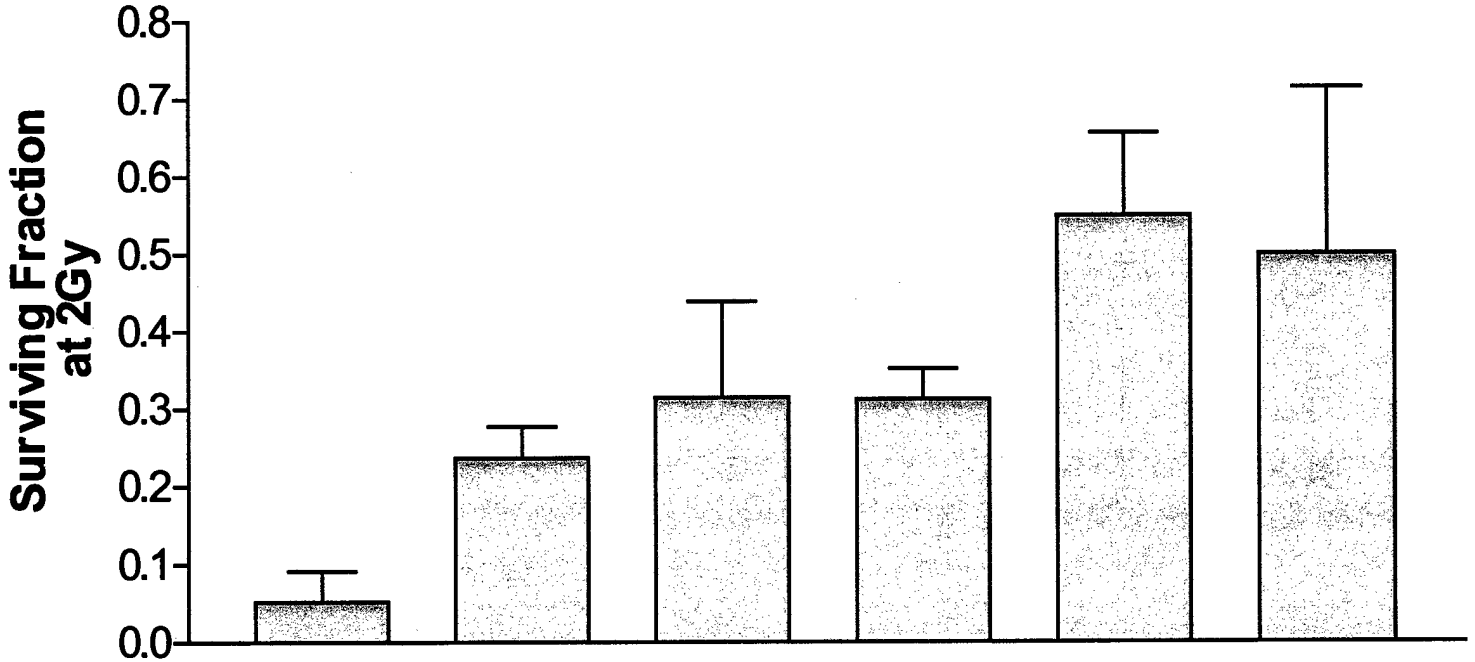
(b)


LNCaP
DU-145
PC-3
10Gy
10Gy
10Gy
NIR 2h 6h 24h NIR 2h 6h 24h 24h
p53
p21WAF1
α-tubulin

(a)

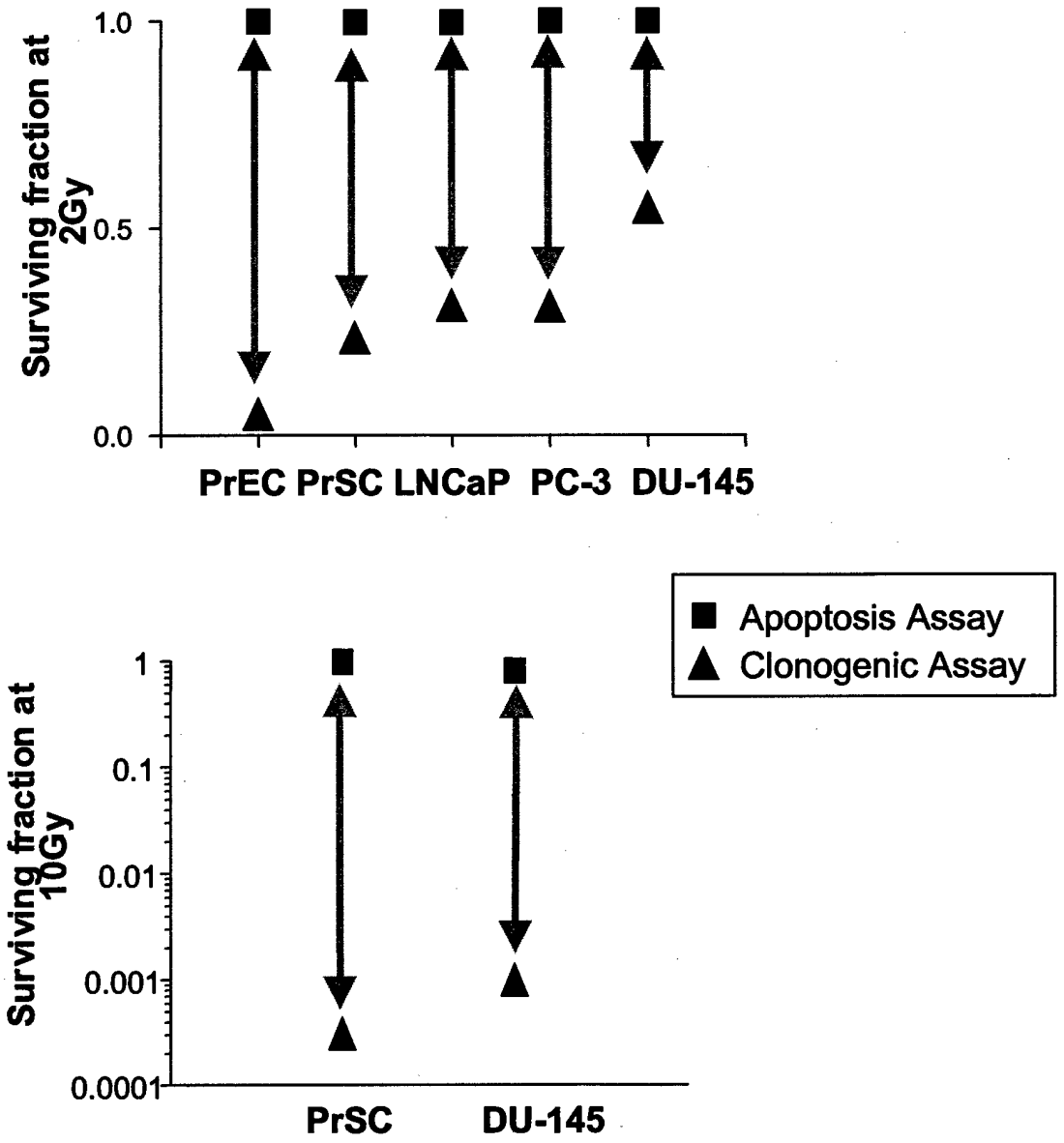

Surviving fraction
1
0.1
0.01
0.001
0.0001
0.00001
0
2
4
6
8
10
Dose (Gy)
Rat-1 HO15.19
DU-145
PrSC
PC-3

(b)

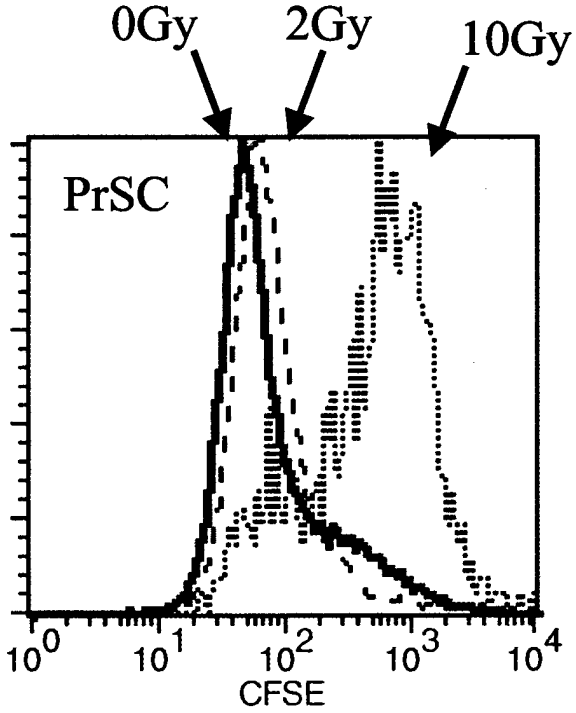


Mean SF2:	0.05	0.24	0.26	0.31	0.55	0.50

(c)


Surviving fraction at 2Gy
1.0
0.5
0.0
PrEC PrSC LNCaP PC-3 DU-145
Surviving fraction at 10Gy
1
0.1
0.01
0.001
0.0001
PrSC
DU-145
Apoptosis Assay
Clonogenic Assay

(a)


0Gy
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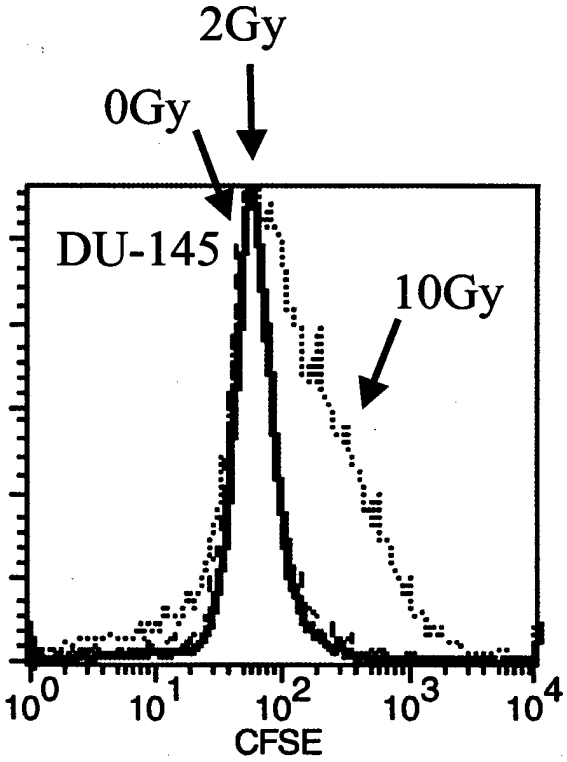
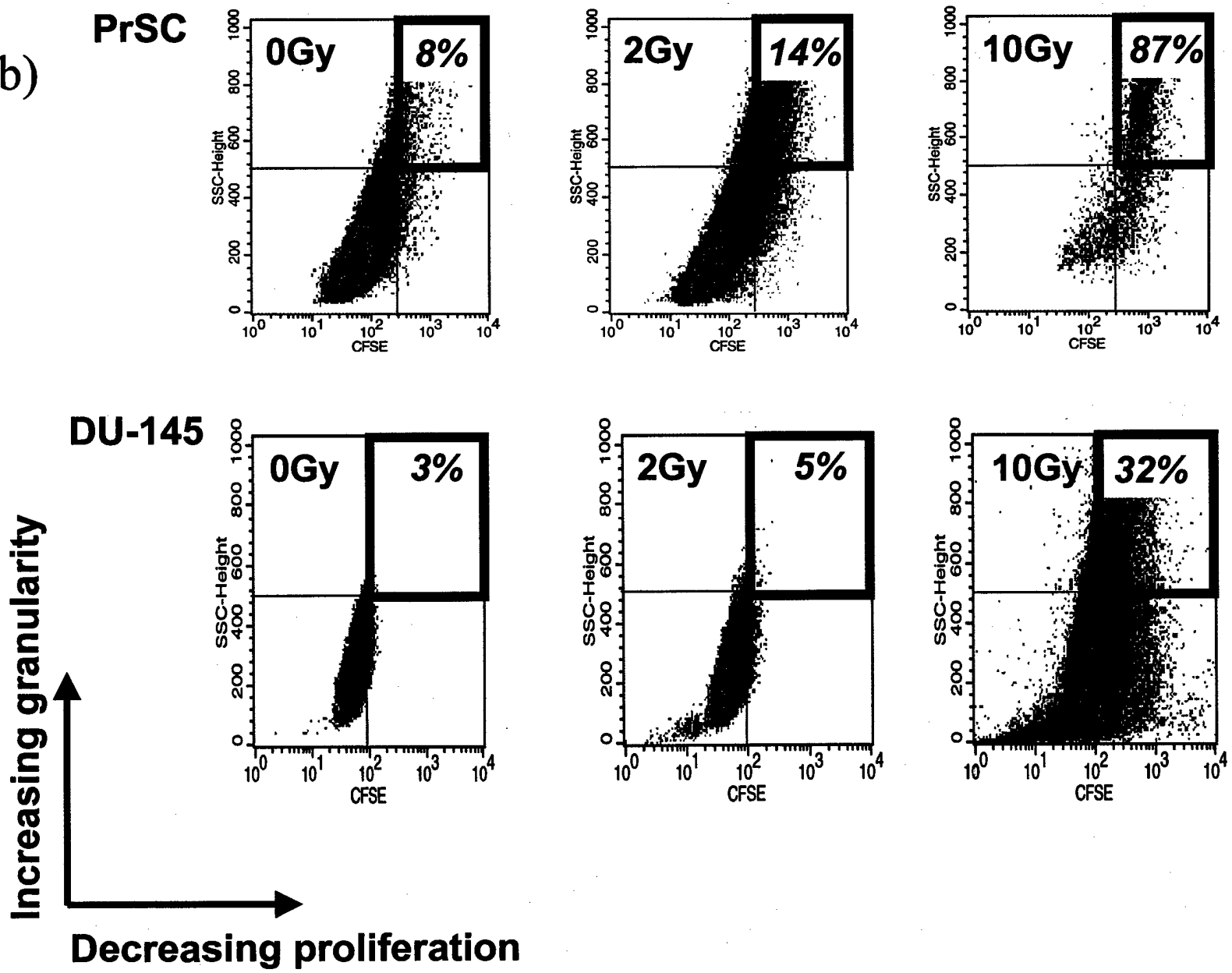
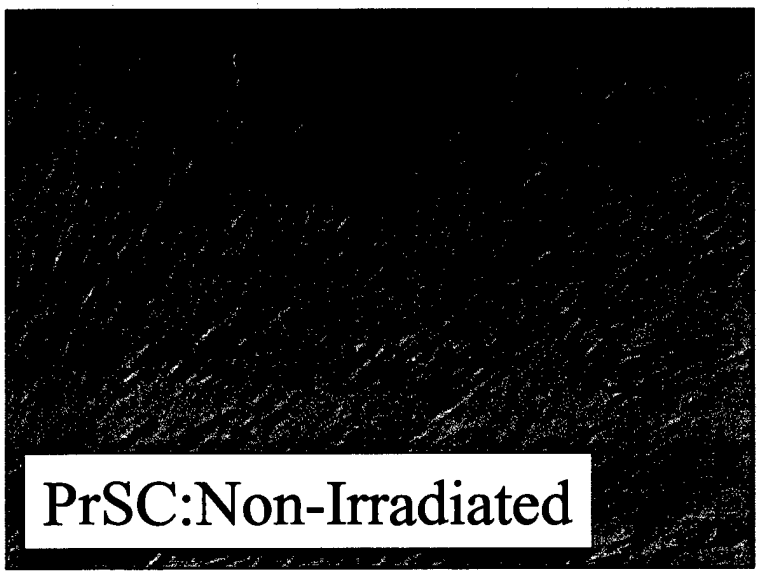
10Gy
PrSC
10^0
10^1
10^2
10^3
10^4
CFSE

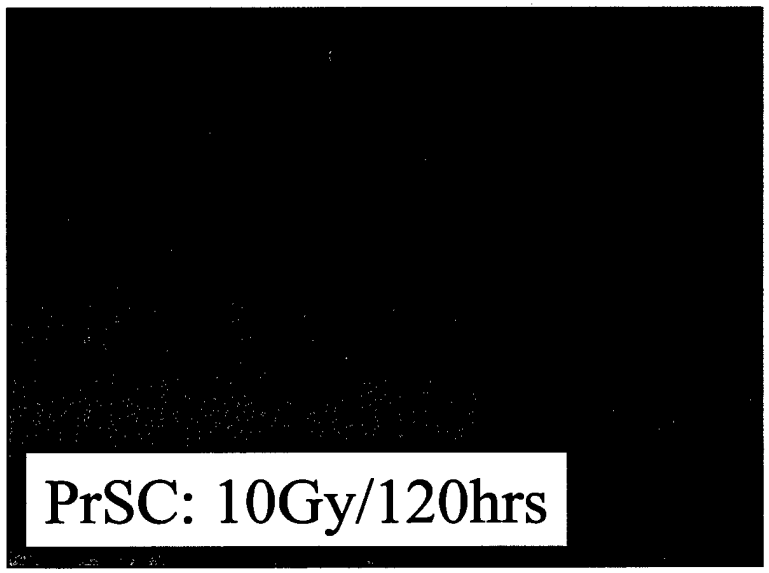

2Gy
0Gy
DU-145
10Gy
10^0
10^1
10^2
10^3
10^4
CFSE

(b)


PrSC
0Gy
8%
2Gy
14%
10Gy
87%
DU-145
0Gy
3%
2Gy
5%
10Gy
32%
SSC-Height
CFSE
Increasing granularity
Decreasing proliferation

(a)





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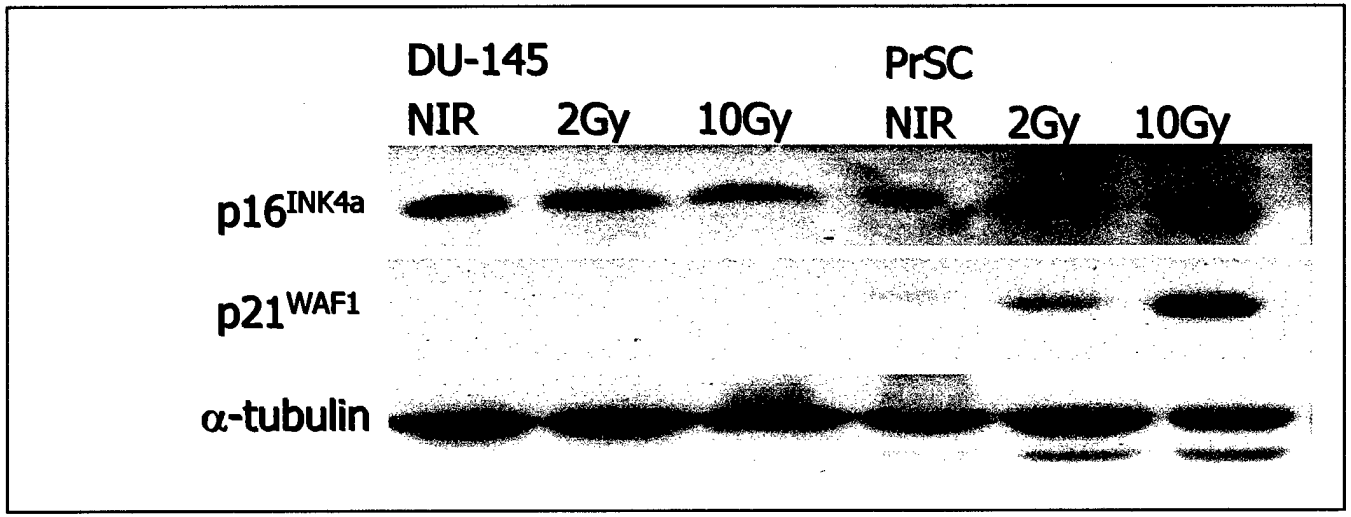
DU-145
PrSC
NIR
2Gy
10Gy
NIR
2Gy
10Gy
p16INK4a
p21WAF1
α-tubulin

CURRICULUM VITAE

PII Redacted

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Date of Last Update: August 2002

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EDUCATION

Post-Graduate Training

1989	Visiting Scientist, Dept. of Experimental Radiotherapy, University of Texas M.D. Anderson Cancer Center, Houston, USA
1990	Summer Research Fellowship, Department of Radiation Medicine, Massachusetts General Hospital, Harvard University, Boston, USA
1993	Internship: Comprehensive Internal Medicine, The Toronto Hospital, Department of Medicine, University of Toronto
1993 -1996	Resident; Department of Radiation Oncology Faculty of Medicine, University of Toronto
1997-1998	Sabbatical-Visiting Scholar, Department of Cell Biology and Genetics Erasmus University, Rotterdam, The Netherlands

Degrees and Licensure

1986-1988	M.Sc., Department of Medical Biophysics (Ontario Cancer Institute) University of Toronto
1992	L.M.C.C. (Canadian General Medical Examinations)
1992	M.D. (Graduated with Honours), Faculty of Medicine, University of Toronto
1993	General Licence; College of Physicians and Surgeons of Ontario
1996	Fellowship of the Royal College of Physicians and Surgeons of Canada (FRCP(C.) Radiation Oncology)
1/1995 -11/1997	Ph.D.; Molecular and Cellular Biology Program Department of Medical Biophysics University of Toronto

APPOINTMENTS

University Appointments:

6/1996-present	Assistant Professor, Department of Radiation Oncology, University of Toronto
11/1998-present	Cross-Appointed Associate Member, Department of Medical Biophysics, University of Toronto

Hospital/Staff Appointments:

6/1996-present	Staff Radiation Oncologist, Princess Margaret Hospital -Part-time practice-completing PhD: PMH-UHN Breast Group: 96/97
11/1998-present	Scientist, Division of Experimental Therapeutics, Research Department, Ontario Cancer Institute
3/1999- present	Full-time practice: PMH-UHN GU Group

ADMINISTRATION/COMMITTEES

Department of Radiation Oncology/Research, PMH-UHN

1994	Department of Radiation Oncology Representative for Association of Residents and Interns of Ontario (PAIRO), Princess Margaret Hospital
1999 – 2002	Secretary, DRO STAFF Monthly Meetings
1999 – present	Member, Postgraduate Education Committee
1999 – present	GU Rounds Organizer, PMH GU-Site Group
2000 - present	Continuing Education Committee, DRO
2000 - present	Section II Leader-Clinical Research Program-Prostate (UHN)
2000 - present	Leader, Clinical Impact Team-Prostate (UHN)

University of Toronto

1990	Member, Palliative Care Subcommittee of the Oncology Coordinating Council, University of Toronto
1991	Member, Aiken's Award Committee on Course Development and Coordination, Faculty of Medicine (Toronto)
1996	Departmental Representative, External Review for Department of Medical Biophysics, University of Toronto
1999 - present	DRO-Departmental Representative, Faculty Council, University of Toronto

Editorial/Review Responsibilities

2000-2001 External manuscript reviewer for:

(i) International Journal of Radiation Oncology, Biology and Physics
(ii) Radiotherapy and Oncology
(iii) Cancer Research
(iv) Journal of Clinical Oncology
(v) Cancer Prevention and Detection
(vi) International Journal of Radiation Biology
(vii) Journal of the National Cancer Institute
(viii) Oncogene
(ix) Carcinogenesis
(x) Cancer letters
(xi) Cancer Epidemiology. Biomarkers and Prevention

Federal/International Granting Bodies - Panels

2000-2003 Panel Member, NCIC Biomedical Awards Panel, Canada
2000 Panel Member, NIH Biomedical Award Study Section, USA
2001(+) Grant Panel Member, Prostate Cancer Research Foundation of Canada
2001 CPCRI IDEA GRANT Panel, May, Toronto
2002 CPCRI IDEA GRANT Panel, June, Toronto
2002 US ARMY DOD Prostate Cancer Research Program Panel, July, Baltimore

Meeting Chair/Co-Chair

2000 Chair; Session on "p53 Strategies", ESTRO Annual Meeting, Istanbul, Sept. 21
2000 Co-Chair; Session on Gene Therapy, ESTRO Annual Meeting, Istanbul, Sept 21.
2001 Chair, DNA Repair II, Radiation Research Society, San Juan, Puerto Rico, April
2001 Chair, Poster Sessions, CARO
2001 Chair, Poster Sessions, ASTRO
2002 Co-Chair, CPC-BioNet National Satellite Meeting, San Francisco, April 2002.
2002 Session Chair, NIH Bio-targeting Workshop, Washington, April 2002.
2002 Chair, Teaching lecture "An introduction to proteomics, ESTRO Annual Meeting, Prague, September 17-21,

Federal/International Panels

2000 Canadian Delegate, NIH Young Investigators Workshop, Bethesda, Maryland
2000 Attendee, Controversies in Prostate Cancer Radiotherapy Meeting, Vancouver, BC
2001 Panel Member, Astra-Zeneca Focus Group on Prostate Cancer, Miami, Jan. 2001
2001 Invited Panel Member, Canadian Urology Association, Prostate Expert Panel
2001 Course Director, UICC Cancer Research Training Course

2002	Member, Scientific Advisory Board, Prostate Cancer Research Foundation of Canada
2001-2002	Chair, National Task Force on Translational Sciences, Canadian Association for Radiation Oncology
2001-2002	ESTRO Scientific Panel Member and Planning Committee

MEDICAL/SCIENTIFIC ORGANIZATIONS - MEMBERSHIPS

American Association for Cancer Research
American Society for Therapeutic Radiology and Oncology
Canadian and Ontario Medical Associations
Canadian Association of Radiation Oncologists
Clinical Research Society of Canada
College of Physicians and Surgeons of Ontario
European Society for Therapeutic Radiology and Oncology
Radiation Research Society
Royal College of Physicians and Surgeons of Canada

SCHOLARSHIPS & AWARDS (since 1994)

1994	W.J. Simpson Award for Resident Research, Department of Radiation Oncology, University of Toronto
1994	1st Prize-Phillips Award for Resident Research CARO-Royal College of Physicians and Surgeons Annual Meeting, Toronto, Canada
1995	Radiation Research Society Travel Award, ICRR, Wurzburg, Germany
1995	Junior Scientist Award, International Congress of Radiation Research Wurzburg, Germany
1995	AACR-Outstanding Poster Award, American Association for Cancer Research Annual Meeting, Toronto, Canada
1995	1st Prize-Phillips Award for Resident Research CARO-Royal College of Physicians and Surgeons of Canada, Annual Meeting, Montreal, Canada
1996	W.J. Simpson Award for Resident Research, Department of Radiation Oncology, University of Toronto
1996	1st Annual Brady Award for Resident Research, Joint Oncology Program, TTH/PMCCC, University of Toronto
1996	University of Toronto Resident Research Award, Canadian Society for Clinical Investigation and Medical Research Council of Canada

ANIMAL PROTOCOL IN USE

2001-2002	Studies in Molecular Carcinogenesis and Molecular Radiobiology in Genitourinary Oncology. University Health Network Animal Care Committee. #99-010.

GRANTS

i) Previous Grants Funded as Principal Investigator:

1998 - 1999

The molecular analysis of mammalian DNA double-strand break repair.
Investigators: **R. Bristow (PI)**
Sponsor: Dean's Research Fund, University of Toronto.
Amount: $9,791.68

1999 – 2000

PMH Foundation Start-up Funds
Amount: $100,000 (one-time award)

1999-2002

Molecular characterization of DNA-dsb repair in transformed cells
Investigators: **R. Bristow (PI)**
Sponsor: National Cancer Institute of Canada, Operating Grant
Amount : $ 320, 360 over 3 years

2000

Molecular and Clinical Aspects of DNA Repair
Investigators: **R. Bristow (PI)**
Sponsor: Canada Foundation for Innovation, New Opportunities Fund
Amount: $386,000 (one-time award; equipment/infrastructure only)

2001-2002

Molecular Radiobiology of Prostate Cancer: Utility and Feasibility of Novel Biomarkers
Investigator: **R. Bristow (PI)**
Sponsor: Prostate Cancer Research Foundation of Canada
Requested Monies: $18,350 over two years

ii) Current Grants Funded as Principal Investigator:

2001-2004

Molecular determinants of radioresponse in prostate cancer
Investigators: **Bristow R(PI),** Warde P, Milosevic M, Lilge L, Squire J, Van Weerden W
Sponsor: United States Department of Defence Prostate Cancer Research
Amount: $292,449 over 3 years

2001

A Molecular Radioresponse Program at PMH-UHN
Investigators: P.Warde **Bristow R (Co-PI)**
Sponsor: GH Woods Foundation
Seed Grant of $50,000 over 2 years

2002

HDAC-Inhibition and Senescence as Potential Radiosensitizers in Prostate Cancer Radiotherapy.
Investigators: **Dr. Robert Bristow (PI),** Dr. Padraig Warde (Co-PI)
Sponsor: Canadian Prostate Cancer Research Initiative (CPCRI) IDEA Grant
Amount: $39,800

2002-2003 Pilot Studies into the Anti-Apoptotic Protein, Survivin, as a Potential Target in Prostate Cancer Radiotherapy.
Investigators: **Bristow R (PI)**, Sweet J, Squire J
Sponsor: Abbot-CARO Uro-Oncologic Radiation Award (ACURA)
Amount: $30,500 yr renewable

iii) Current Grants Funded as Co-Investigator/Collaborator:

2000-2001 Molecular genetic mechanisms of prostatic oncogenesis: opportunities for new therapies and treatment options. Project II: Predicting individual treatment response
Investigators: **Bristow R**, Milosevic M, Tsihlias J
Sponsor: Princess Margaret Hospital Cancer Research Program
Amount: $70,000 in year 1

2000-2001 Implementing prostate core biopsies during radiotherapy to determine genetic and microenvironmental biomarkers relevant to prostate cancer radioresistance and metastatic spread
Investigators: **Bristow R**, Milosevic M, Hedley D, Warde P, Khokha R
Sponsor: Princess Margaret Hospital Cancer Impact Team
Amount: $114,000 in year 1

2001-2004 A Study of Transrectal Tumor Oxygen Measurements in Patients with Clinically Localized Prostate Cancer
Co-Investigator (Translation al DNA-protein studies);
Investigators: M. Milosevic (PI), Toi A, Sweet J, **Bristow R**, Hedley D, Panzarella T, Hill R
Sponsor: US Army Department of Defence Prostate Research Program
Amount: 105, 000/yr

2001-2006 A Micronutrient and Molecular Prostate Cancer Canadian Network
Investigators: Fleshner N, **Bristow R (Co-Administrator)**, Gleave M, Rennie P, Klotz L, Khokha R, Squire J, Pollak M
Sponsor: NCIC-Canadian Prostate Cancer Research Initiative Program Grant
Amount: $666,000/yr x 5 years renewable

2002 Equipment maintenance support for oncologic molecular micro-imaging (OMM)
PI: Ming-Sound Tsao
Co-applicants: **R. Bristow**, S. Done, D. Hedley, R. Hill, M. Ikura, R. Khokha, J. Squire, B. Wilson, S. Wong, G. Bradley.
Amount: $ 48,000 per year x 3 years

iv) Clinical Trials as PI or Co-PI

1999-2004

Phase III study of neoadjuvant hormonal therapy in patients with localized prostate cancer treated with escalated dose radiotherapy
Investigators: Warde P, Catton C, Milosevic M, **Bristow R**, Catton P, Crook J, Gospodarowicz M, McLean M, Panzarella T
Sponsor: AstraZeneca
Amount: $150,000

REB# 01-0555-C

2001

A Pilot Study of Pre-Operative Conformal Radiotherapy in 15 Patients Treated With Radical Prostatectomy – PMH Protocol
Investigators: **Dr. Robert. Bristow (PI),** Drs. P Warde (Co-PI), N. Fleshner (Co-PI)

REB# 01-0483-C

2000

Influence of tumor hypoxia on outcome following radiotherapy, and on prostate cancer malignant progression – PMH Protocol
Investigators: Milosevic M (PI), Toi A, Sweet J, **Bristow R**, Hedley D, Panzarella T, Hill R.

REB#00-0443-C (Dr. Milosevic)
REB#01-0620-C (Dr. Bristow)

PUBLICATIONS

Refereed Publications

(i) Published:

1. **Bristow R**, Jang A, Peacock J, Chung S, Benchimol S, Hill RP: Acquired radioresistance in REF transformants expressing mutant p53 protein. Radiat Res 41:121-123, 1995.

2. Peacock J, Chung S, **Bristow R**, Hill R, Benchimol S: The p53-mediated G1 checkpoint remains intact in tumorigenic REF clones transformed by HPV16-E7 and EJ-ras. Mol Cell Biol 15(3):1446-1454, 1995.

3. **Bristow R,** Brail L, Jang A, Peacock J, Chung S, Benchimol S, Hill RP: P53-mediated radioresistance does not correlate with metastatic potential in tumorigenic REF cell lines following oncogene transfection. Int J Radiat Oncol Biol Phys 34(2):341-355, 1996.

4. **Bristow R,** Benchimol S and Hill RP: The p53 gene as a modifier of intrinsic radiosensitivity: implications for radiotherapy. Radiother Oncol 40: 197-223, 1996

5. **Bristow R.**, Laperriere N, Tator C, Milosevic M, Wong CS: Post-operative radiotherapy for dermoid tumours of the spine: a report of 3 cases. J Neurooncol 33:251-256, 1997

6. Reitmar A, Risley R*, **Bristow R***, Wilson T*, Ganesh A, Jang A, Peacock J, Benchimol S, Hill RP, Fishel R, Mak TW, Meuth M: Mutator phenotype in Msh2 deficient mouse embryo fibroblasts. (*1st four authors contributed equally) Cancer Research 57: 3765-3791, 1997.

7. **Bristow RG**, Hu Q, Jang A, Chung S, Peacock J, Benchimol S, Hill R: Radioresistant MTp53-expressing rat embryo cell transformants exhibit increased DNA-dsb rejoining during exposure to ionizing radiation. Oncogene 16(14): 1789-1802, 1998.

8. Warde P, O'Sullivan B, **Bristow RG**, Panzarella T, Keane TJ, Gullane PJ, Witterick IP, Payne D, Liu FF, McLean M, Waldron J, Cummings BJ: T1/T2 glottic cancer managed by external beam radiotherapy: the influence of pretreatment hemoglobin on local control. Int J Radiat Oncol Biol Phys 41(2): 347-353, 1998.

9. Jalali, F., Al-Rashid, S., Lilge, L. and **R. Bristow**; DNA Repair-related Protein Foci in Irradiated Human Fibroblasts: Preliminary Correlates to DNA-dsb rejoining and Radiosensitivity. Radiotherapy and Oncology 57: S35-38, 2000.

10. Milosevic M, Toi A, Sweet J, **Bristow R**, Warde P, McLean M, Crook J, Catton C, Catton P, Gospodarowicz M. Trans-rectal oxygen measurements in prostate cancer. Clin Invest Med 23 (Supp), S19, 2000.

11. Parker C, Milosevic M, Toi A, Sweet J, Panzarella T, Syed A, **Bristow R**, Catton C, Catton P, Crook J, Gospodarowicz M, Maclean M, Warde P, Hill R A polarographic electrode study of tumor oxygenation in localized prostate cancer; (Conditionally accepted, Radiotherapy and Oncology, 2002)

12. Gangopadhyay S, Jalali F, Reda D, Peacock J, **Bristow R** and Benchimol S: Expression of different mutant p53 transgenes in neuroblastoma cells leads to different cellular responses to genotoxic agents. Exp Cell Res 275(1):122-131, 2002.

13. Atsushi Hirao, Alison Cheung, Gordon Duncan, Andrew J. Elia, Andrew Wakeham, Hitoshi Okada, Talin Sarkissian, Jorge A. Wong, Takashi Sakai, Elisa de Stanchina, **Robert G. Bristow,** Penny A. Jeggo, Scott W. Lowe, Stephen J. Elledge and Tak W. Mak. Chk2 selectively regulates p53-mediated apoptosis in an ATM-independent manner (In press, Molecular and Cellular Biology, 2002).

14. Bromfield G, Fan R, Meng A, Kumaravel Ts, **Bristow RG**. Radiation-Induced Death Pathways in Prostate Cells: Role of Apoptotic and Non-Apoptotic Cell Death (In press, Prostate Cancer and Prostate Diseases, August 2002).

15. Cheung AMY, Prakash Hande M, Jalali F, Ming-Sound T, Skinnider B, Hirao A, McPherson JP, Karaskova J, Suzuki A, Wakeham A, Itie, A, Elia A, Squire J, **Bristow R**, Hakem R, Tak WM, Chromosomal aberrations and enhanced p53-dependent tumorigenesis in Brca2-deficient T cells (Conditionally accepted, Cancer Research, 2002).

Manuscripts in Preparation

1. Al Rashid S T, Jalali F, Meng A, Lilge L, Benchimol S, Mak T and **Bristow RG**. the Ser15-phosphorylated form of the p53 protein: a sensor of DNA damage *in vivo*.

2. Jalali F, Al Rashid S, Meng A and **Bristow R**. DNA-dsb Rejoining in Fibroblast Strains of Differing p53 Status: Defect in Li-Fraumeni Syndrome?

3. **Bristow RG**, Peacock J, Jang A, Hill RP and Benchimol S: Radioresistance in murine ras-transformed cells due to gain of MTp53 function.

Refereed Book Chapters

1. **Bristow R**, Hill RP: Molecular and Cellular Basis of Radiotherapy. In: The Basic Science of Oncology (3rd edition), ed I Tannock and RP Hill, McGraw-Hill Ltd.; New York, 1998.

2. Gospodarowicz MK, Warde P, **Bristow RG**: Definitive Radiation Therapy in Bladder Cancer. In: The Clinical Management of Bladder Cancer, ed R. Hall, 1999

3. Gospodarowicz, MK, O'Sullivan and **Bristow RG**: Host and Tumor-related Prognostic Factors, in UICC Handbook of Prognostic Factors; Chapter 6, pp. 71-94; (ed. MK Gospodarowicz) Wiley-Liss Inc., USA, 2001.

4. **Bristow, RG**, Blussyen, H., de Klein, A. and van Gent, D. The PI3-Kinase family as Sensors and Signals for DNA Damage Responses; in Trends in Molecular Biology; Chapter 6, pp. 71-94; (ed. MK Gospodarowicz) Wiley-Liss Inc., USA, 2001.

5. Milosevic M, Gospodarowicz M, Jewett M, **Bristow R**, Haycock T. Intensity-modulated radiotherapy for lymph node metastases in bladder cancer. In: Gregoire V, Scalliet P, Ang KK (Eds). Clinical Target Volumes in Conformal and Intensity-Modulated Radiation Therapy. 2002.

Non-Refereed Publications

1. **Bristow R:** Checks and balances: cell-cycle control and radiation oncology. Current Oncol 3(2):1-7, 1996

2. **Bristow R**, Benchimol S, Hill RPL: P53: protein expression or protein function ? Re: Awwad et al., IJROBP 34(2):323-332, 1996. Int J Radiat Oncol Biol Phys 35 (5):1123-1124, 1996 (letter).

Abstracts Published & Presented

(1) **Bristow R**, Sukhdeo, M. and D. Mettrick. "The effects of trichinella spirella infection on the crypt cell production rate in Wistar rats" Presented at the American Physiological Society, Niagara Falls, USA, October 1985.

(2) **Bristow R**. and R.Hill. "In Vitro Prediction of Radioresponse and Predictive Assays". Prediction of Tumour Treatment Response Conference, Banff, April 1987.

(3) **Bristow R**. and R.Hill. "In Vitro Prediction of Radioresponse and Predictive Assays". Radiation Research Meeting, Philadelphia, April 1988

(4) **Bristow, R**. and R.Hill. "In Vitro Prediction of Radioresponse and Predictive Assays". Clinical Research Society of Toronto, April 1989.

(5) **Bristow, R**, Savin, S., Hittelman, W. and W. Brock. "Chromosomal Damage Following XRT and 5-FU in CHO Cells". Presented at Radiation Research Society, New Orleans, April 1990.

(6) Pardo, F., Kley, N., Fucarelli, A., **Bristow, R.**, et al." Biologic and Cell Kinetic Studies on primary and transformed rat embryo fibroblasts" Presented at the annual meeting of the American Society of Therapeutic Radiation Oncology, Miami, USA, October 1990.

(7) **Bristow, R.**, Hunt, T. and F. Pardo. "Ras and radioresistance". International Congress of Radiation Research, Toronto, July 1991.

(8) **Bristow, R**, Peacock, J., Benchimol, S. and R. Hill. "Effects of ras and mutant p53 on radioresistance and metastasis". National Medical Student Research Forum, Galveston, April 1991.

(9) **Bristow, R.**, Peacock, J., Benchimol, S. and R. Hill. "Effects of ras and mutant p53 on radioresistance and metastasis". Symposium of Genetics of Radioresistance, Montreal, July 1991.

(10) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "Mutant p53-expression dependent changes in radioresistance in a REF transformant model" American Association of Cancer Research Special Conference on Perturbations in Cell Cycle Control and Genomic Stability, Banff, February 1994.

(11) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, A., Benchimol, S. and Hill, R.P. "Rat embryo cells transfected with a mutant p53 gene show increased radioresistance" Annual Meeting of the Radiation Research Society, Memphis, April 1994

(12) Peacock, J., Chung, S., **Bristow, R.G.**, Hill, R.P., Benchimol, S. "The p53-mediated G1 checkpoint remains intact in rat mebryo fibroblasts transfected with ras or HPV16-E7." The 7th International p53 Workshop, Muskoka, Ontario, June 1994.

(13) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "Acquired radioresistance in cells expressing a mutant p53 protein". Gene Induction and Adaptive Responses in Irradiated Cells: Mechanisms and Clinical Applications, Montreal, June 1994.

(14) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. " Mutant p53 increases radioresistance in transfected rat embryo fibroblasts." Annual Meeting of the Royal College of Physicians and Surgeons of Canada, CARO session, Toronto, September 1994.

(15) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "Mutant p53 increases radioresistance in rat embryo fibroblast cell models". Annual Meeting of the American Society for Therapeutic Radiology and Oncology, San Francisco, October 1994.

(16) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "MTp53-mediated radioresistance in rodent tumour cells: Dominant-negative mechanism or gain of function?". Annual Meeting of the American Association for Cancer Research, Toronto, April 1995

(17) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "Mechanisms of MTp53-mediated radioresistance in rodent tumour cell models". Annual Meeting of the Royal College of Physicians and Surgeons of Canada, CARO session, Montreal, September 1995

(18) **Bristow, R.G.**, Peacock, J., Chung, S., Jang, S., Benchimol, S. and Hill, R.P. "Mechanisms of MTp53-mediated radioresistance in rodent tumour cell models". International Congress of Radiation Research, Wurzburg, August 1995

(19) **Bristow, R.G.**, Peacock, J., Jang, A., Penn, L., Hill, R.P., Benchimol, S. "Role of the c-myc gene in tumour progression following loss of p53 gene function". Annual meeting of the Royal College of Physicians and Surgeons of Canada, CARO session, Halifax, September 1996.

(20) **Bristow, R.G.**, Jang, A., Peacock, J., Hill, R.P., Benchimol, S. "Radioresistant REF transformants expressing MTp53 are proficient in DNA dsb rejoining". Gordon Research Conference: "Molecular Biology of Radiation Oncology", Plymouth, New Hampshire, June 1997.

(21) **Bristow, R.G.**, Jang, A., Peacock, J., Hill, R.P., Benchimol, S. "Increased DNA-dsb rejoining in tumour cells expressing MT-p53 protein", Poster: 21st Annual Meeting of the American Radium Society, Monte Carlo, May 1998.

(22) **Bristow, R.G.**, Jang, A., Peacock, J., Hill, R.P., Benchimol, S. "Increased DNA-dsb rejoining in tumour cells expressing MT-p53 protein", Poster: Annual Meeting of Royal College of Physicians and Surgeons of Canada, Toronto, September 1998.

(23) **Bristow, R.G.**, Jalali, F. Shim, K. Warde, P., and Benchimol, S. "Molecular Mechansims of Radioresistance in Prostate Cancer", Poster; 3rd World Congress on Uorological Research, Paris, France, September-October, 1999.

(24) "DNA Repair-related Portein Foci in Irradiated Human Fibroblasts: Preliminiary Correlates to DNA-dsb Rejoining and Radiosensitivity", ESTRO-MITRE Conference, Brussels, Belgium, Dec, 2000.

(25) Jalali F, **Bristow, R G.** "Characterization of DNA-DSB Repair in Fibroblasts With Altered p53 Status", Radiation Research Society, San Juan, Puerto Rico, April 2001.

(26) Bromfield, G P, **Bristow, R G.** "Radiation-Induced Cell Death Pathways in Normal and Malignant Prostate Epithelial Cells", Radiation Research Society, San Juan, Puerto Rico, April 2001.

(27) Al Rashid S T, Jalali F, Lilge L, **Bristow R G.** "Ser15-phosphorylated p53: a Sensor of DNA-damage Following Ionizing Radiation", Radiation Research Society, San Juan, Puerto Rico, April 2001.

(28) Al Rashid S T, Jalali F, Lilge L, **Bristow R G.** "The p53 Protein as a Direct Sensor of DNA-Damage in Human Cells Following Ionizing Radiation", CARO Meeting, Quebec City, September 2001(abstract accepted).

(29) Bromfield, G P, **Bristow, R G.** "Relative Importance of Non-Apoptotic and Apoptotic Cell Death Pathways in Irradiated Prostate Cells", CARO Meeting, Quebec City, September 2001 (abstract accepted).

(30) Al Rashid S T, Jalali F, Lilge L, **Bristow R G.** "Ser15-phosphorylated p53 as a Sensor of DNA-damage Following Ionizing Radiation", ESTRO/ECCO Meeting, Lisbon, Portugual, October 2001 (abstract accepted).

(31) Al Rashid S T, Jalali F, Lilge L, **Bristow R G.** "Ser15-phosphorylated p53 as a Sensor of DNA-damage Following Ionizing Radiation", ASTRO Meeting, San Francisco, November 2001 (abstract accepted).

(32) Bromfield G, Fan R, Meng A, Kumaravel Ts, **Bristow R G.** "Radiation-Induced Death Pathways in Prostate Cells: Role of Apoptotic and Non-Apoptotic Cell Death", American Association for Cancer Research, San Francisco, April 2002.

(33) Al Rashid S T, Jalali F, Lilge L, **Bristow R G.** "Ser15-phosphorylated p53 as a Sensor of DNA-damage Following Ionizing Radiation", Radiation Research Society, Reno Nevada, April 2002

(34) **Bristow R. G.**, Bromfield G, Fan R, Meng A, Kumaravel TS. "Radiotherapy-induced death pathways in prostate cells: Apoptosis versus permanent cell cycle arrest", 57th annual CUA Meeting, Newfoundland, June 2002. (abstracted accepted)

(35) Parker C, Panzarella T, Catton C, **Bristow R**, Crook J, Gospodarowicz M, McLean M, Michael Milosevic M and Warde P "The Effect of Haemoglobin Level on Biochemical Outcome following Radiotherapy in Localised Prostate Cancer", CARO, Toronto, 2002.

(36) **Bristow RG**, Fan R, Kumaravel TS, Bromfield G, Jalali F, Meng A. "DNA Repair in normal and malignant prostate epithelial tissues: implications for genetic stability and radiotherapy", ESTRO, Prague, September 2002.

TEACHING DOSSIER

A) POSTGRADUATE MEDICAL EDUCATION

1) Resident Training – Department of Radiation Oncology (Toronto)

1994	Department of Radiation Oncology, University of Toronto 1) "Management of acute airway and superior vena caval obstruction"(PGY2) 2) "Understanding the 4 R's of radiotherapy" (PGY3-4) 3) "The molecular biology of cancer with respect to radiotherapy" (PGY5)
1996	1) PGY2 Resident teaching session, Radiation Oncology, University of Toronto -"Management of acute airway and superior vena caval obstructions" 2) Resident Planning Drills - November 1996
1999	Resident rotation teaching: Dr. Jennifer Knox (Medical Oncology) RCPSC Mock Drill: Andrew Bailey (Radiation Oncology) Mock Examiner: DRO-Resident End of Year Planning Exams (Drs. Ian Poon, and Andrew Loblaw)
2000-2001	Senior DRO Resident's Tutorial Sessions-"Clinical Radiobiology" 4 x 2 hours Junior DRO Residents Tutorial-"Molecular Radiobiology" 1 x 2 hrs
2002	(Residents) Medical Oncology Teaching. "Essential Elements of Radiation Oncology" x1hr – January 2002.
2002	Junior DRO Residents (PGY1 & 2) Tutorial – Radiobiology, "Molecular Radiobiology" Part I & II" 2 x 2hrs – January & February 2002.
2002	DRO Resident – Dr. Jackie Spayne
2002	DRO Fellow - Dr. Iain Ward
2002	DRO Fellow - Dr. Andrew Coleman

2) Resident Training – External/National

1997-2001	Lecturer, Applied Radiobiology Resident Seminar "Molecular Biology, University of British Columbia and Washington
2000	University of Western Ontario, London, Ont. (8 hrs lecture) Radiobiology Review Course, January, 2000

B) SCHOOL OF GRADUATE STUDIES, UNIVERSITY OF TORONTO

1) Course Lecturer/Director:

1996-1997	MBP 1018Y, Department of Medical Biophysics, University of Toronto (2 Hours each): "Radiobiology and Breast Cancer"; "Radiation Response in Lymphoma and Leukemia"
1999–2001	MBP 1008, University of Toronto: Fundamentals in Cell Biology II (4 x 2 hr lectures in 2000; 1 x 2 hours in 2001; exam marking)
2001-2002	Course Director, MBP1018Y, Designed and administrated Course; 5 x 2 hr lecturers, set and marked examinations

2) Department of Medical Biophysics Supervised:

(i) Summer Students:
a) Katherine Shim (Summer 1999)
b) Wissam Assaily (Summer 1999)
c) Harshna Patel (Summer 2001 & 2002)

(ii) Graduate Students:
MSc candidate – Gillian Bromfield (1999-2002; graduated)
PhD Candidate – Shahnaz Al-Rashid (1999-current; NCIC Studentship)
MSc Candidate – Dr. Andrew Coleman (2002-present)

(iii) Post-Doctoral Fellows:
Dr. R. Fan (January, 2001-current)
Dr. T. Kumaravel (April, 2001-current; CPCRI-NCIC Fellow)

(iv) Student committee member of following students (start-date in parentheses):
2000-M.Sc - Lynn Shepherd (Supervisor: Dr. Ian Tannock)
2000-PhD - Victor Yang (Supervisor: Dr. Brian Wilson)
2000-MSc. - Patrica Ruozo (Supervisor: Dr. David Hedley)
2000-PhD - Rebecca Gladdy (Supervisor: J. Danska/c. Guidos)
2001-M.Sc. - Irina Matei (Supervisor: J. Danska/C. Guidos)
2001-M.Sc. - Jorge Wong (Supervisor: Dr. K. Vallis)
2001-M.Sc. - Wissam Assaily (Supervisor: Dr. Benchimol)
2001-M.Sc. - Phillip Wong (Supervisor: Dr. I. Tannock)
2001-M.Sc. – Jennifer Lau (Supervisor: Dr. D. Hedley)
2001-M.Sc. – Richard Chahwan (Supervisor: Dr. Hakem)
2001-M.Sc. – Carlos Rendon (Supervisor: Dr. Lothar Lilge)
2002-MSc – Carol Lee (Supervisor: Dr. Malkin – HSC)

C) UNDERGRADUATE – UNIVERSITY OF TORONTO

1) Individual Teaching:

1996-1997	2 University of Toronto Medical Students; Clinical teaching x 1 month each
1998	University of Toronto 4th year B.Sc. Student (Bodour Salhia), PSL497F, Department of Physiology, University of Toronto-basic science mentor
10/1998-10/1999	University of Toronto BSc. in Radiation Services 1) Predictive Assays (1 hr lecture) 2) Molecular Radiobiology (1 hr lecture)
1999	Medical Student Teaching/Rotation: Greg Czarnotta, University of Toronto
2000	Medical Student Teaching/Rotation: David Robertson, University of Toronto
2002	Medical Student Teaching: Danny Vespirini (4th year student)
2002	Medical Student Teaching: Alan Andrew (4th year, Ivan Smith Scholarship)

2) Course Lecturing:

2000	Course Lecturer – University of Toronto, ANA 401S, Cancer Biology (2 x 2 hr lectures; exam and written proposal marking)
2001	Med School: Molecular Mechanisms in Cancer I and II (Pathobiology Course), Faculty of Medicine, November 2001.

Continuing Medical Education (CME) MEETINGS ATTENDED:

1988	CME-Pain Symposium, Medico-Legal Society, University of Toronto
1989	CME-HIV Infection, Mt. Sinai Hospital, University of Toronto
1991	CME: Update in Haematology/Oncology, Mayo Clinic, Scottsdale, AZ
1993	CME: Clinical Trials in Medicine, University of Toronto
1994	CME: Update in Radiation Oncology; University of Toronto
1994	CME Workshop on Methods in Medical Education/Teaching, TSRCC, University of Toronto
1995/1998	CME Workshop: Genito Urologic Oncology, University of Toronto
1999	CME Update: Trends in Radiation Oncology, University of Toronto
2002	CME Workshop on UroOncology,

LOCAL/NATIONAL/INTERNATIONAL ROUNDS PRESENTED

1995	Grand Rounds, PMCCC "P53 and therapeutic response"
1995	University of Toronto Department of Radiation Oncology Rounds-"Primer on Molecular Radiobiology"
1997	PMCC Breast Group Rounds, "P53 as a determinant of Therapeutic Response in breast cancer: fact or fiction ?
1997	Research Rounds, Medical Genetics Centre, Erasmus University Rotterdam, Bristow, R.G., von Gent, D. "Molecular mechanisms of non-homologous recombination in DNA dsb repair following ionizing radiation" , Oct 13, 1997
1998	Leiden University, The Netherlands. Joint Erasmus University-Leiden University MGC Rounds, "Micro-homology Usage as a Component of DNA-dsb repair", May, 1998.
1998	Erasmus University, Rotterdam. Departmental Research Rounds. "Linear DNA Constructs as Probes for DNA-dsb Repair Pathways". June, 1998.
1999	Experimental Therapeutics Seminar, Molecular Mechanisms of DNA-dsb repair", OCI, February, 1999.
1999	DRO-PMH Rounds: "DNA-DSB Repair: A discrete or indiscrete affair?" April/99.
1999	"Conformal radiotherapy", Oshawa Urologic Surgeons Meeting; sponsored by Zeneca, May/99.
2000	DRO-PMH Rounds, "Genetics of Connective Tissue Disorders: Relationship to DNA repair?"

2002 UHN Prostate Cancer Training Course, "Individualizing Prostate Cancer Radiotherapy: Focusing on Genetics Rather than Politics", PMH April/02

VISITING PROFESSORSHIPS/INVITED LECTURES

(1) "P53 as a determinant of therapeutic response: fiction or function" ? Department of Radiation Oncology Visiting Professor, University of Pennsylvania, Philadelphia, December 17, 1996.

(2) "P53 protein as a modifier of DNA repair", Invited Lecture, Dept. of Genetics, University of British Columbia, Nov. 1997.

(3) "Apoptosis vs. Necrosis in Cancer Research", Invited Lecture, NCIC Palliative Care Meeting, Vancouver, B.C. , Nov. 1997.

(4) "P53 as a determinant of therapeutic response", Invited Lecture, CERRO/ESTRO Meeting, Les Menuires, France, Jan. 1998.

(5) "P53 and radioresistance: A role for DNA repair?" Invited Lecture, Department of Experimental Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands, May 1998.

(6) "P53 and radioresistance: A role for DNA repair?" Invited Lecture, Department of Radiation Oncology and Radiobiology, Catholique University, Brussels, Belgium, June 1998.

(7) "P53 and radioresistance: A role for DNA repair?" Invited Lecture, Department of Experimental Oncology, University of Aarhus, Aarhus, Denmark, May 1998.

(8) "Increased DNA-dsb rejoining in tumour cells expressing MT-p53 protein", Invited Lecture, Second Wolfsberg Conference in Molecular Radiobiology, Ermatingen, Switzerland, May 1998.

(9) "Mammalian DNA Double-Strand Break Repair: A Discrete, or Indiscrete, Affair?", Invited Lecturer, Molecular Biology Refresher Course, American Society of Therapeutic Radiation Oncology, New York, January 15-16th, 1999.

(10) "P53 and DNA-dsb repair", Invited Lecture, CERRO/ESTRO Meeting, Les Menuires, France, Jan. 1999

(11) "G1 Cell Cycle Checkpoint and DNA Damage Response", Invited Review Lecture; Future of Radiobiology in Radiation Oncology International Symposium, Department of Radio-Oncology, June, 1999, University of Essen, Germany.

(12) Invited Speaker/Debator: Annual Debate: CME Course in Trends in Radiation Oncology, Univeristy of Toronto, Toronto. May 1999

(13) "MTp53 Increases Cell Survival By Increasing DNA-dsb rejoining", Invited Lecture, 1st Annual Mid-West DNA Repair Meeting; University of Michigan, May, 1999.

(14) Visiting Professor, " Genetics Determinants of Radiation Curability", Department of Radiation Oncology, University of British Columbia, February 2000.

(15) "Modifying DNA repair capacity as genetic target for radiotherapy", Invited Lecture, First International Conference on Translational Research and Pre-Clinical Strategies in Radio-Oncology, Lugano, Switzerland, March 5-8th, 2000.

(16) "p53 as therapeutic target", Invited Lecture, First International Conference on Translational Research and Pre-Clinical Strategies in Radio-Oncology, Lugano, Switzerland, March 5 – 8th, 2000

(17) "P53-DNA damage and repair responses" Invited Speaker/Review, European Society for Therapeutic Radiation Oncology, Istanbul, Sept. 21, 2000.

(18) " DNA-dsb Repair Foci: Implications for DNA Biomarkers: Invited Speaker, ESTRO-CERRO Annual Meeting, Les Menuires, France, March 2001

(19) "The p53 tumour suppressor protein and DNA repair: new tricks for the old dog?": Invited Speaker, University of Pennsylvania Medical Center, April 2001

(20) "The p53 tumour suppressor protein and DNA repair: new tricks for the old dog?" Invited Speaker, University of Ottawa, April 2001

(21) "DNA Repair and Carcinogenesis" UICC Cancer Research Training Course, Invited Speaker and Course Director, Toronto, May 2001

(22) "New Aspects of Radiation Oncology in Prostate Cancer", Astra-Zeneca Update on Urology, Invited Speaker, Deerhurst, Ontario, May 2001

(23) "Molecular Biomarkers in Radiation Oncology", Target Insight DRO-CME Meeting; Invited Speaker; May 2001

(24) "The p53 tumour suppressor protein and DNA repair: new tricks for the old dog?" Invited Speaker, Amgen Scientific Retreat, Collingwood Ontario, April 2001

(25) "Combined Modality Therapy for Prostate Cancer"; Invited Speaker, Canadian Association of Urology Annual Meeting, Toronto, June, 2001

(26) "New techniques in external beam radiation treatment for prostate cancer" Invited Speaker, Bierstock Family Symposium, Toronto, September 2001.

(27) "The New Genetics and Radiation Oncology: Defining a Molecular Therapeutic Ratio" Invited Speaker, Cobalt 50th Anniversary in London, Advances in Radiation Therapy Symposium, London, Ontario, October 2001.

(28) Bristow R. "Novel Biomarkers of DNA Damage Responses: Implications and Limitations for Predicting Radiotherapy Response", Invited Speaker, NIH Bio-targeting Workshop. Washington, April 2002.

FIGURE 1:

Neutral comet assay results show increased residual DNA breaks at 24 hours following irradiation (10 and25 Gy; aerobic, RT) in malignant prostate cells in comparison to normal repair kinetics in normal prostate cells. Similar findings were observed utilizing the alkaline comet assay.

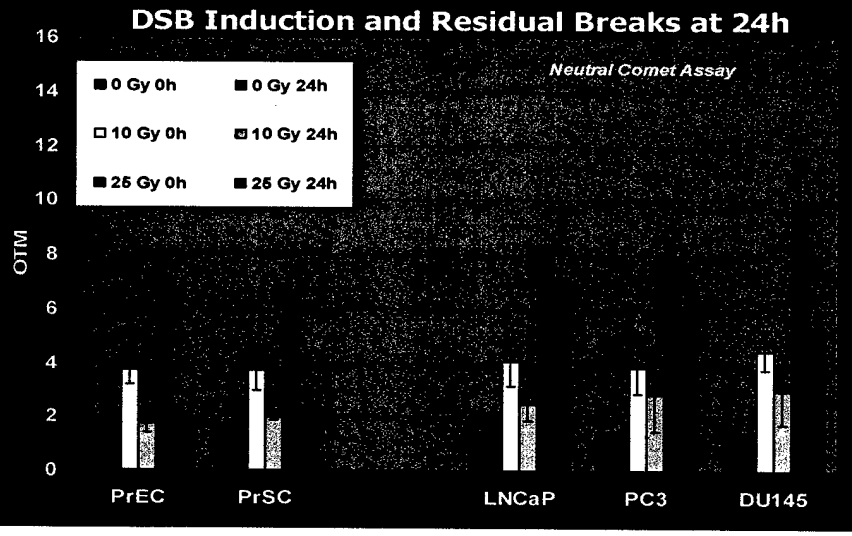

Figure 2a

RNA Protection Analyses sugges increased basal level of transcription for homologous recombination genes involved in DNA repair in malignant cell lines compared to normal cell lines. Error bars represent SE.

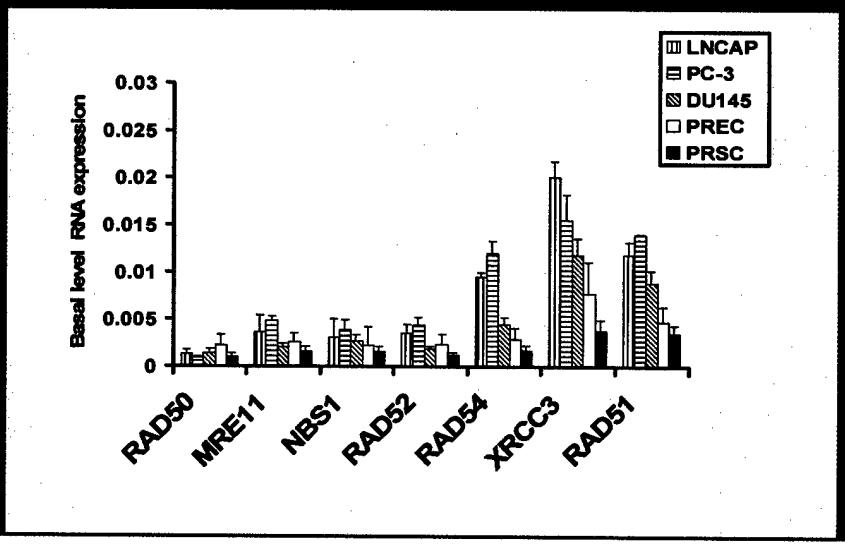

Figure 2b

Increased HR-Associated Protein Expression in malignant prostate cell lines compared to tubulin loading controls; these results corelated to RNA overexpression profile seen in Figure 2.

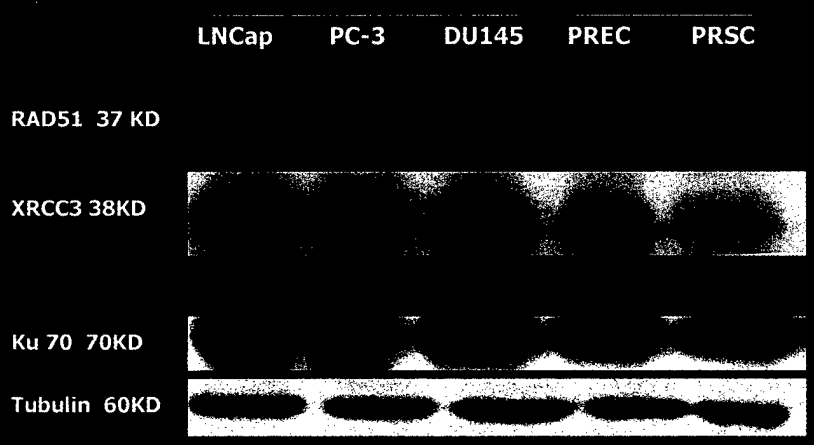

Figure 2c

Increased HR-Associated Protein expression in malignant prostate cell lines based on densitometry of Western Blot analyses shown in Figure 2b with b-tubulin as loading control

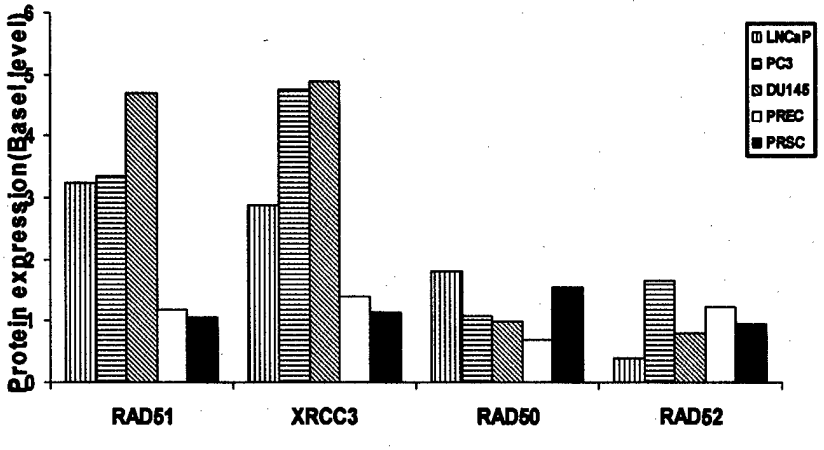

Figure 3a

Positive control for irradiation-induced nuclear foci: Formation of gamma-H2AX protein is normal; dose-responsive, in normal and malignant prostate cell lines. Cells were stained with antibodies to H2AX post 10Gy XRT at 4 hrs; counterstained with DNA-DAPI stain.

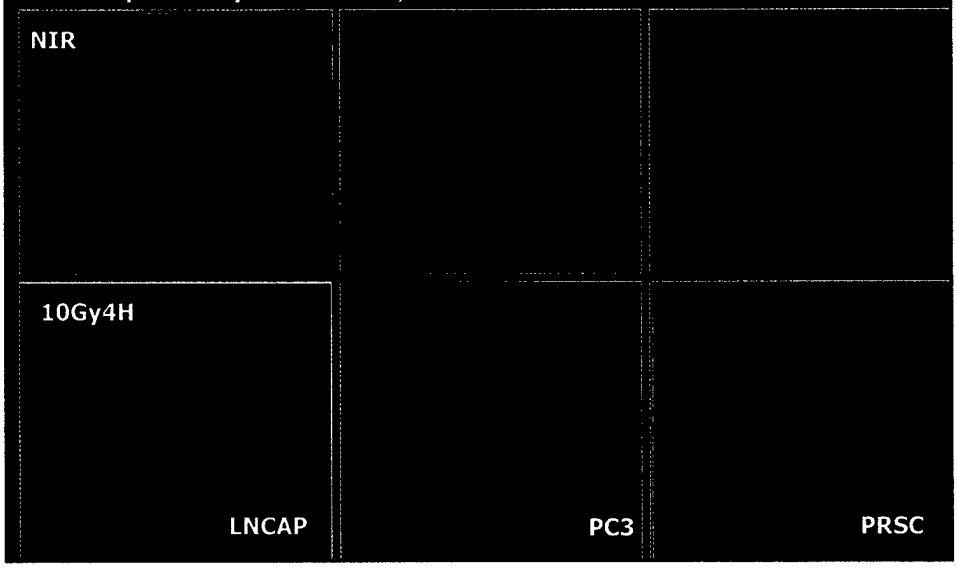

Figure 3b

Rad51 foci formation is defective following XRT (same conditions as in Figure 3a) in malignant LNCaP, PC-3 And DU-145 cells and is not dose-responsive; evidence for nulcear transport defect

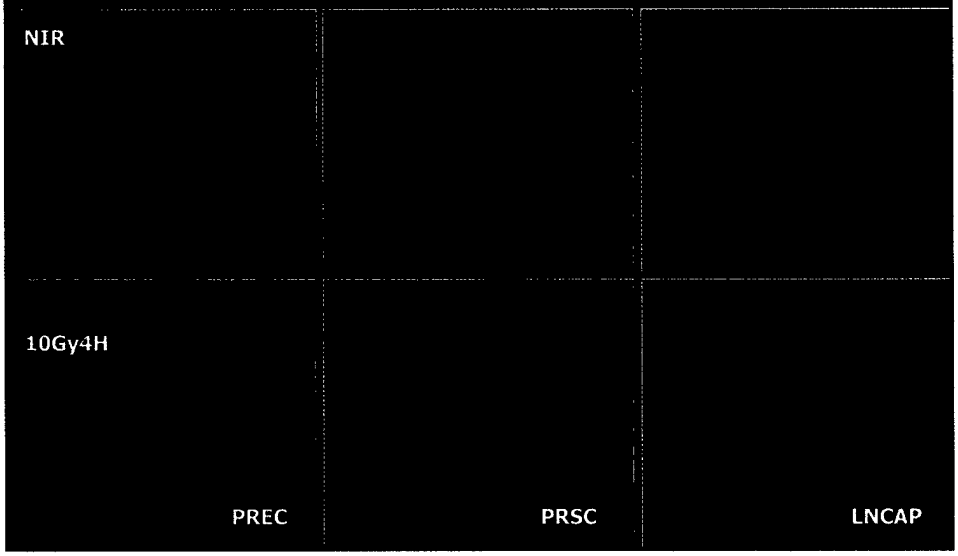

Figure 3c

Quantitation of Data shown in Figure 3b: Decreased IRIF Rad51 Nuclear Response Following 10 Gy at 4 hours in malignant prostate cell lines

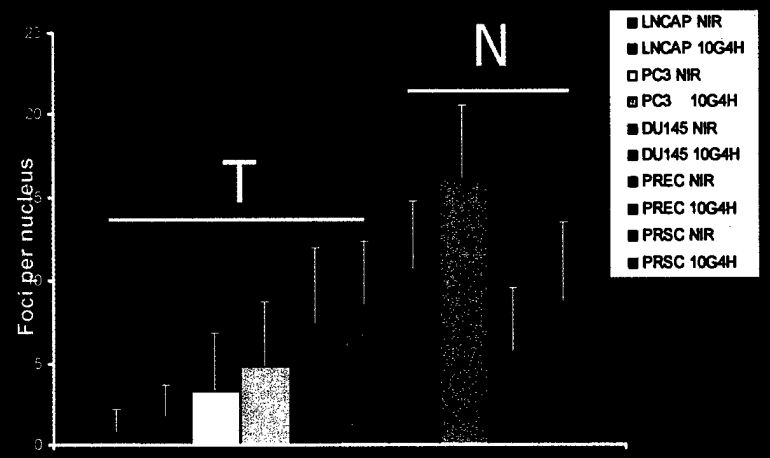

Figure 4

Immunohistochemistry for HR-associated repair proteins in PC-3 xenografts growing in nude mice and irradiated with 20 Gy; removal of tissues at 24 hours post irradiation and formalin fixation. Staining for rad51 and BRCA2 proteins shows increased intensity psot-XRT. Similar results were observed for rad50.